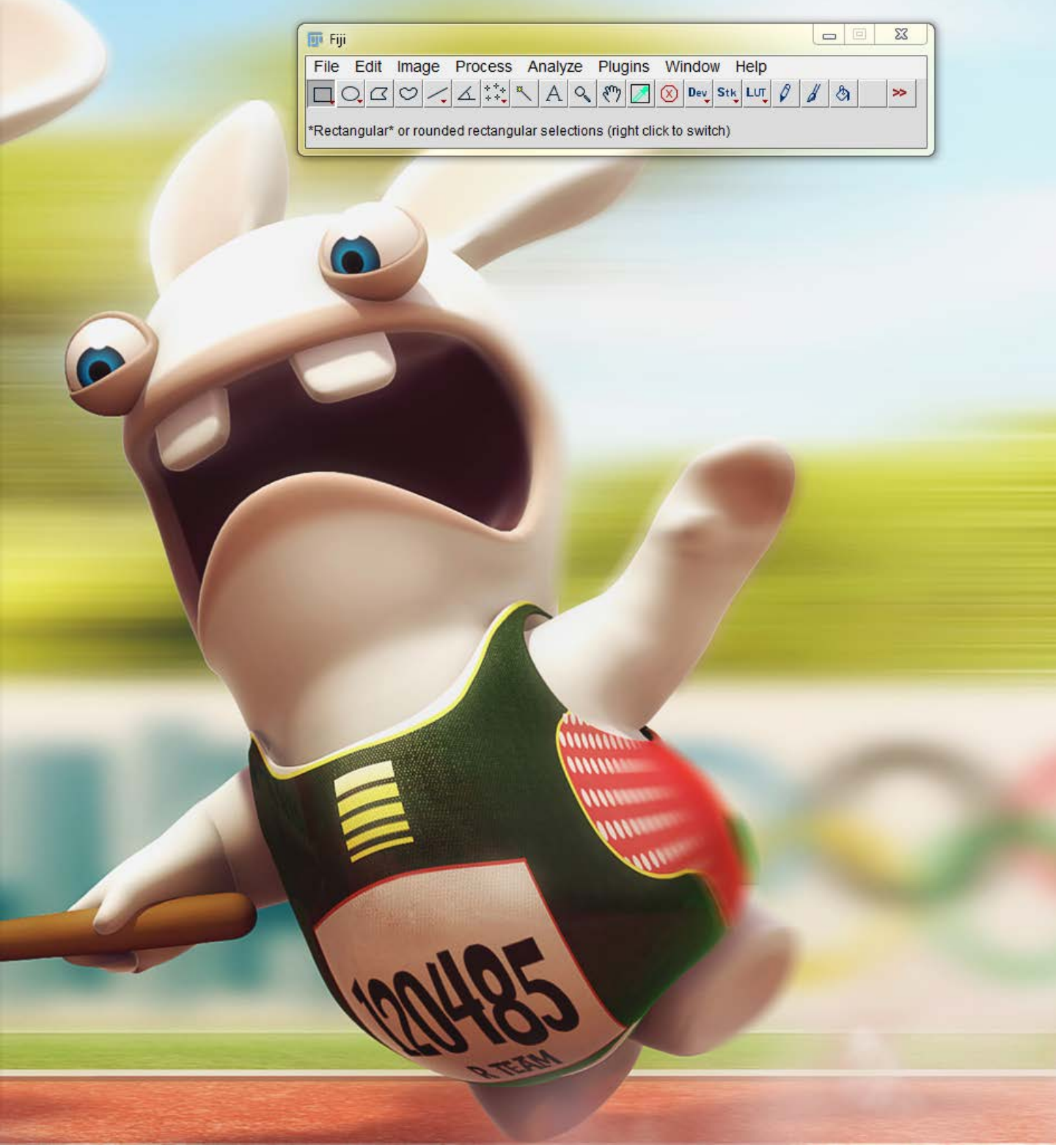
AIS_quant manual

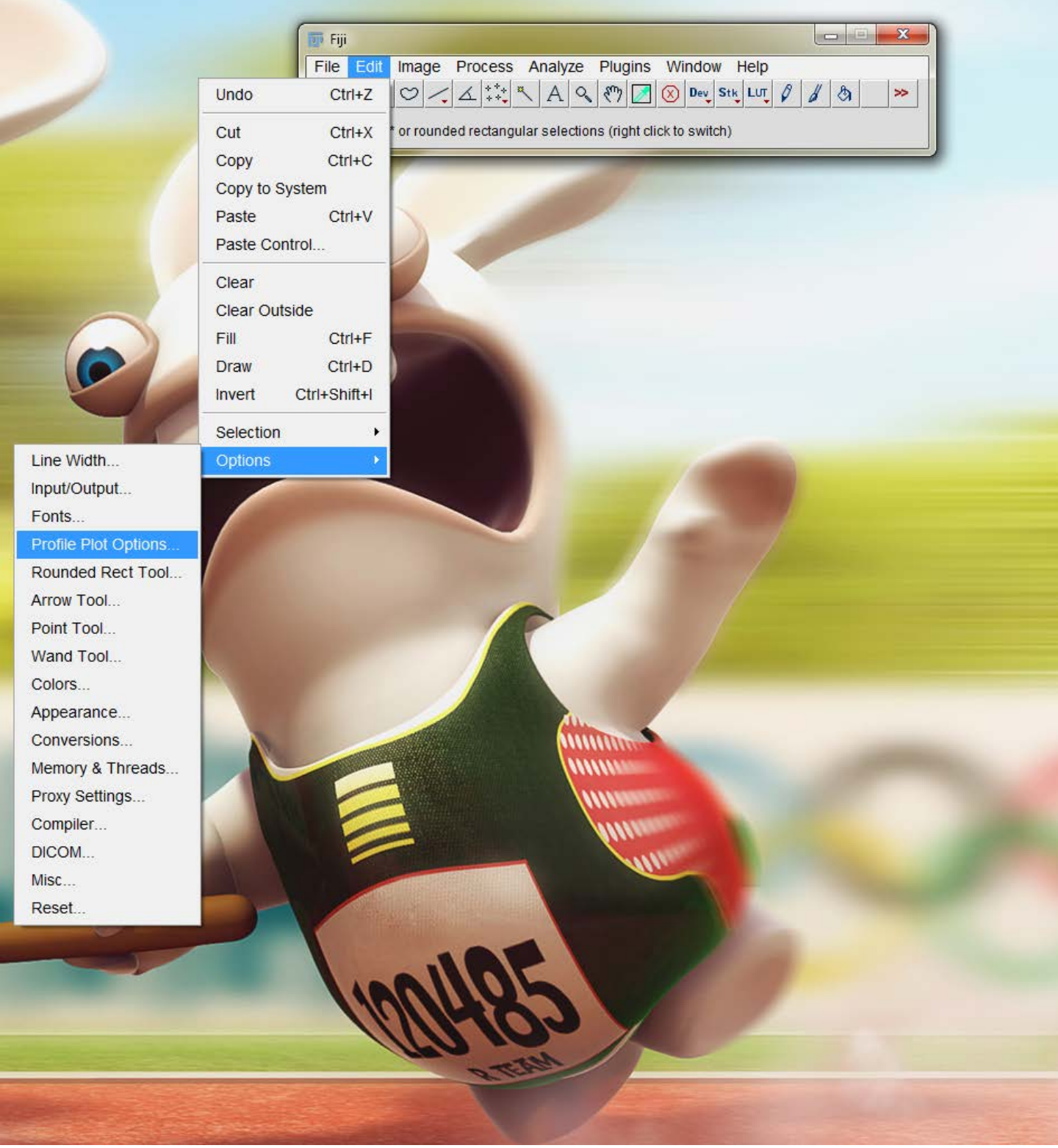
Version 1.0
2012-11-26
Sam van Beuningen

Please read before use: M. S. Grubb, and J. Burrone, 'Activity-Dependent Relocation of the Axon Initial Segment Fine-Tunes Neuronal Excitability', *Nature,* 465 (2010), 1070-4.

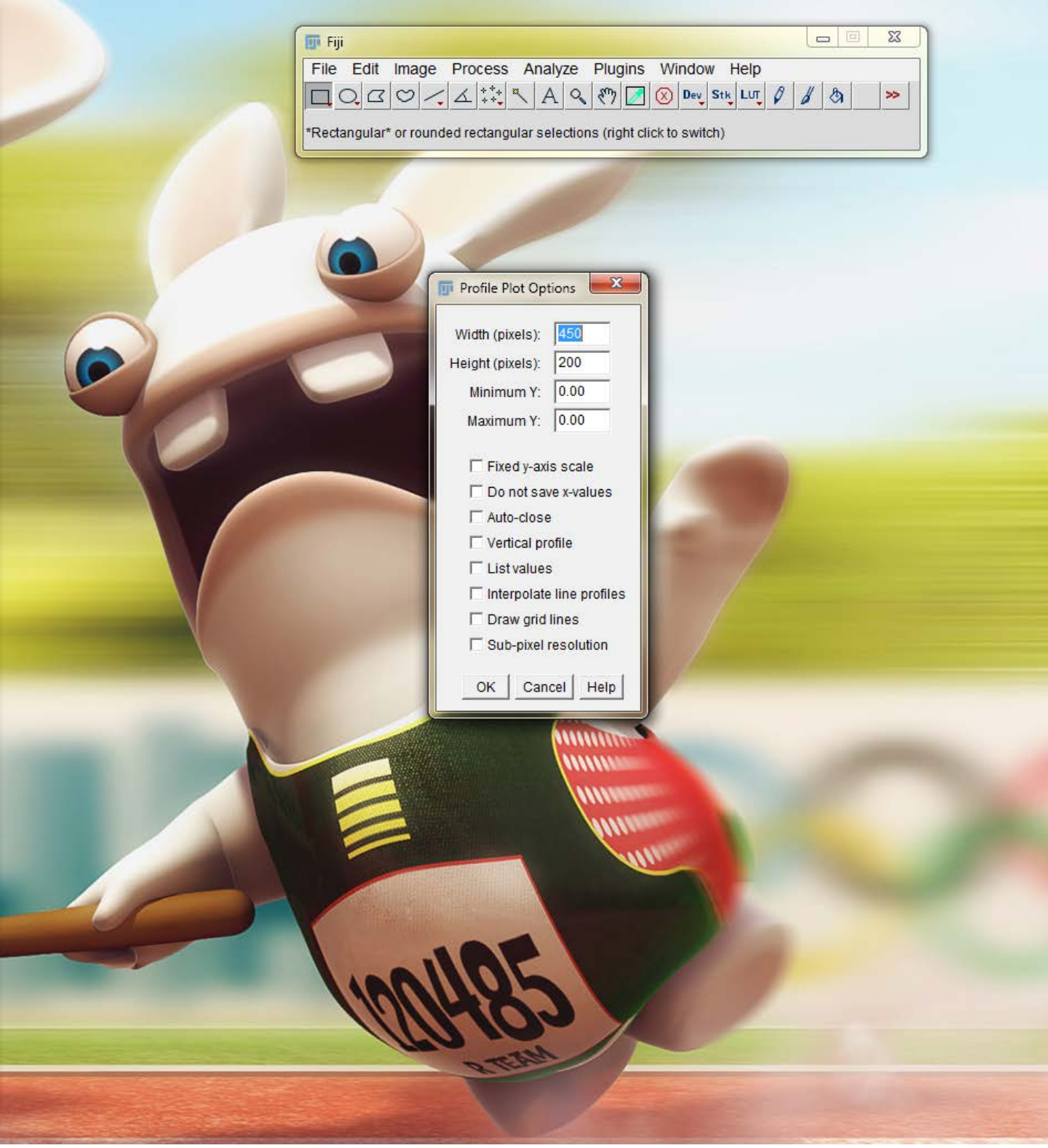
Many thanks to Eugene Katrukha



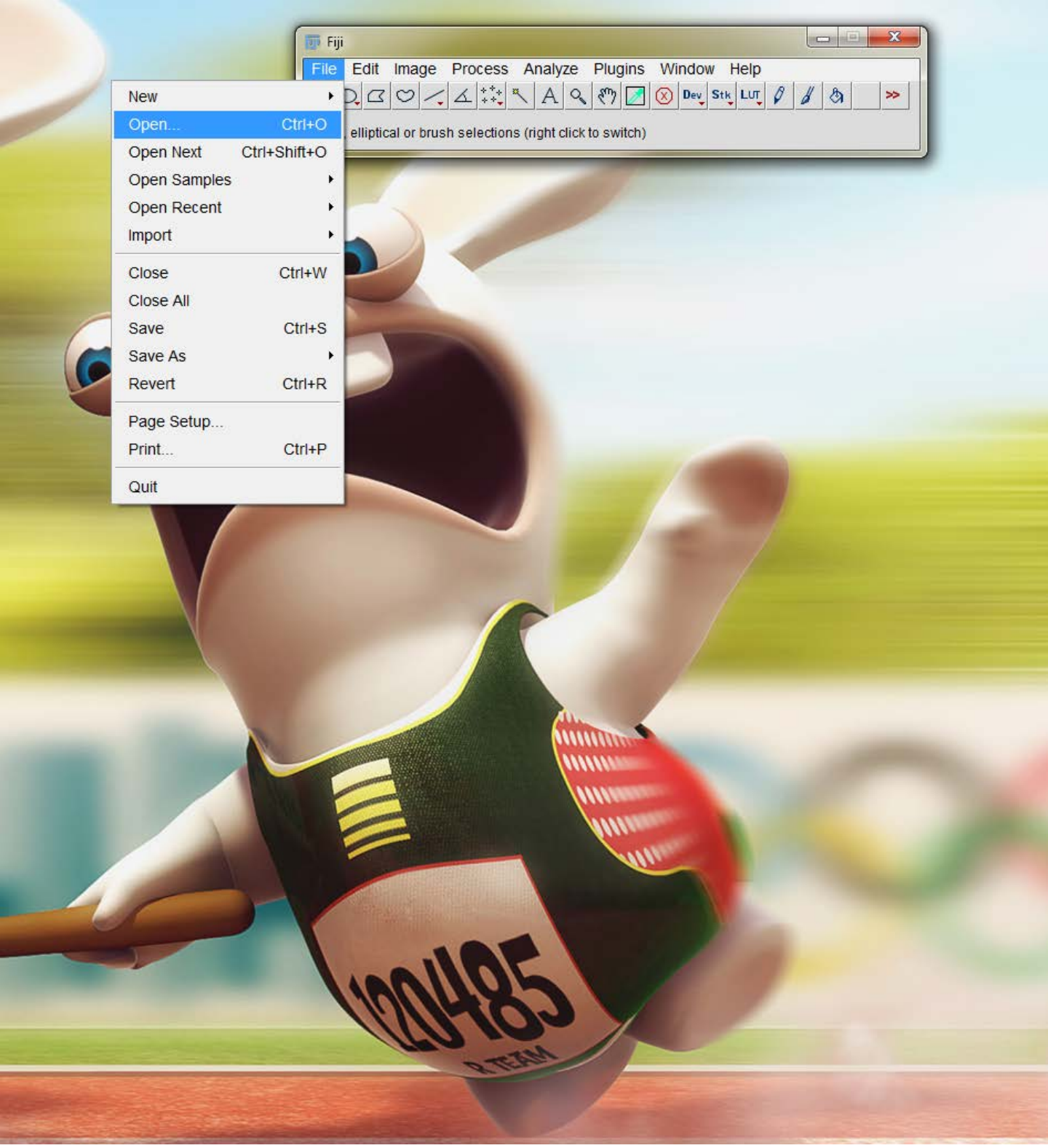
- Open ImageJ or Fiji



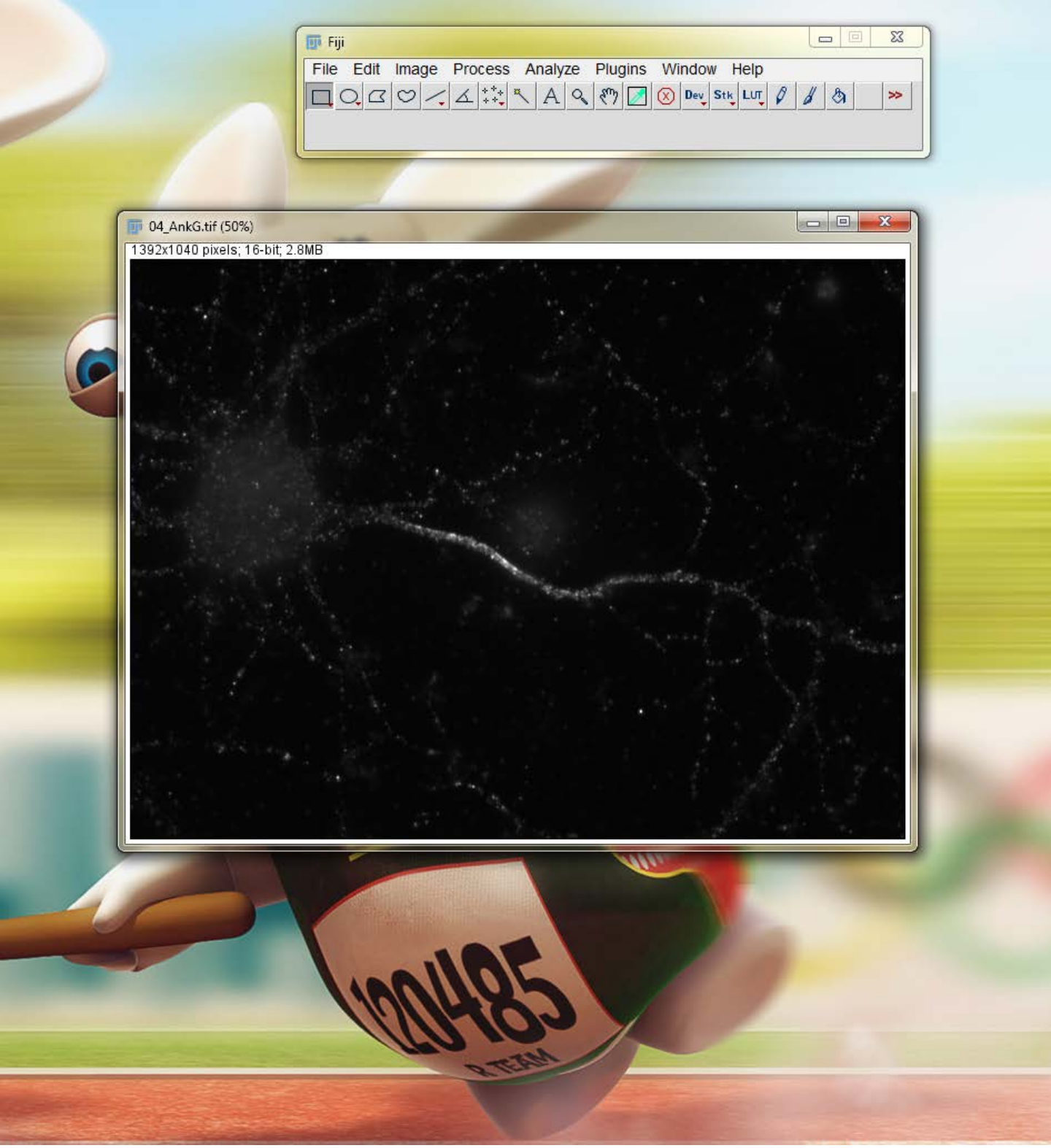
- Check Profile Plot Options



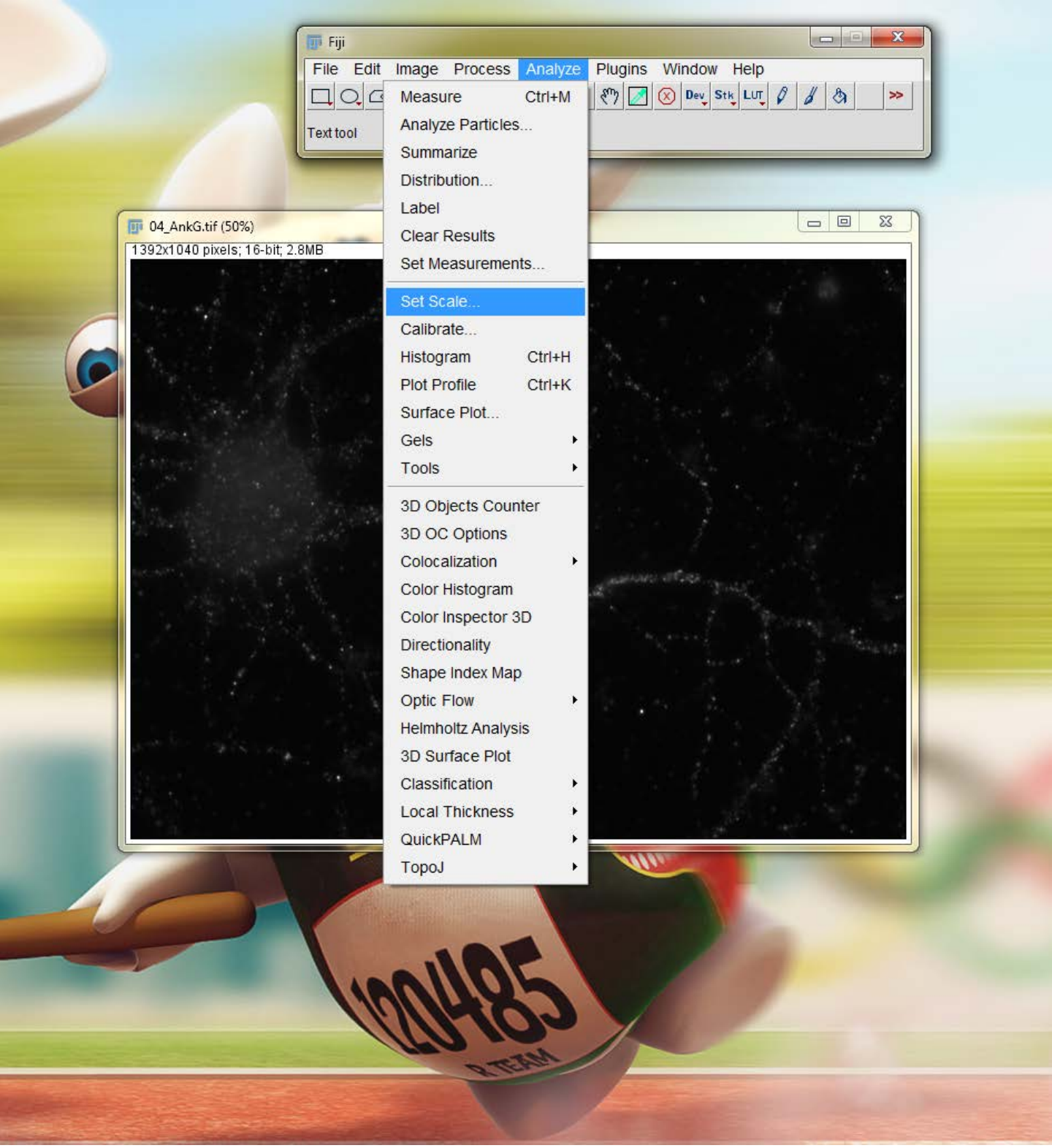
- Be sure that everything is unchecked



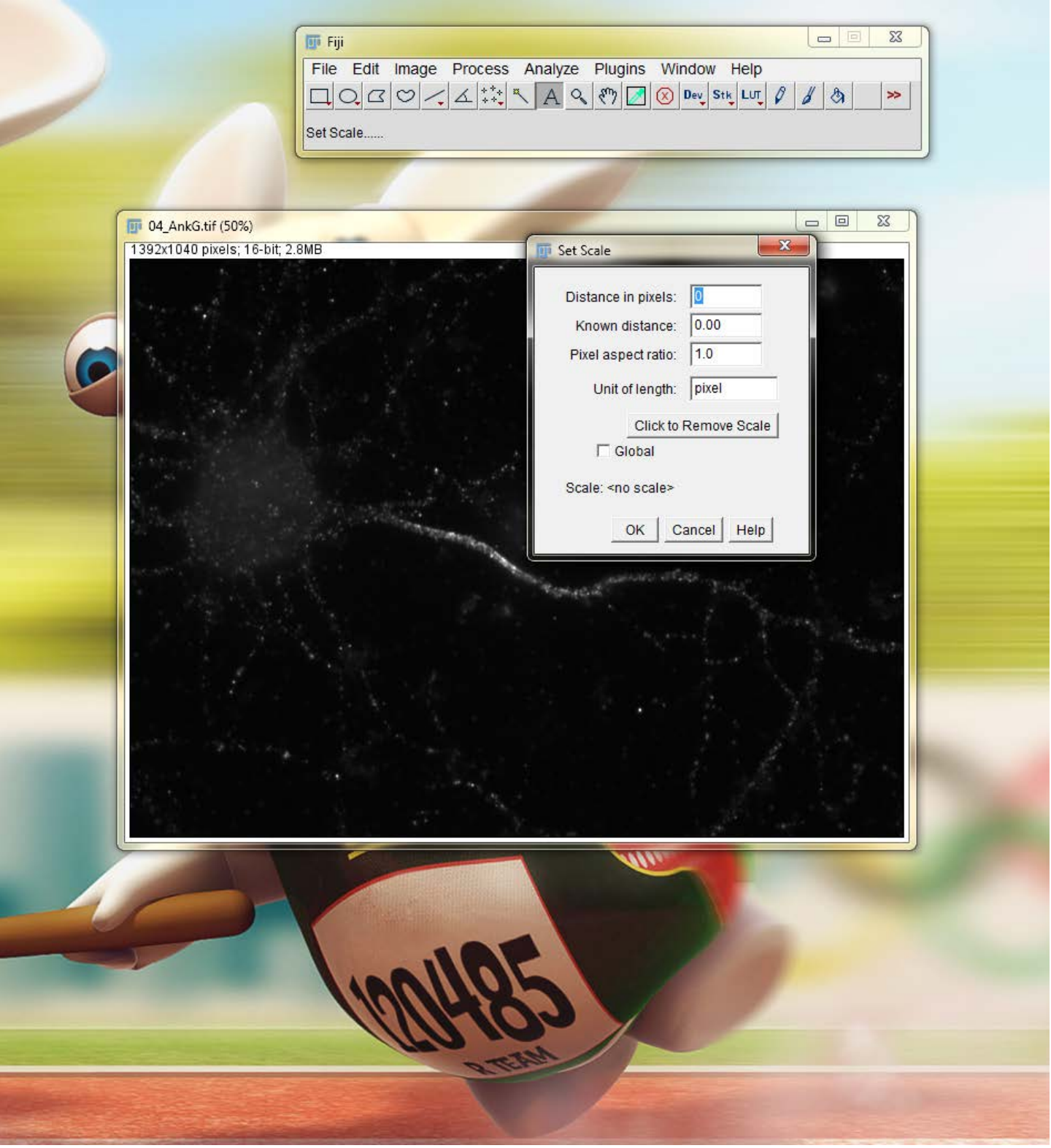
- Open a picture with your staining (you can also drag it into the program).



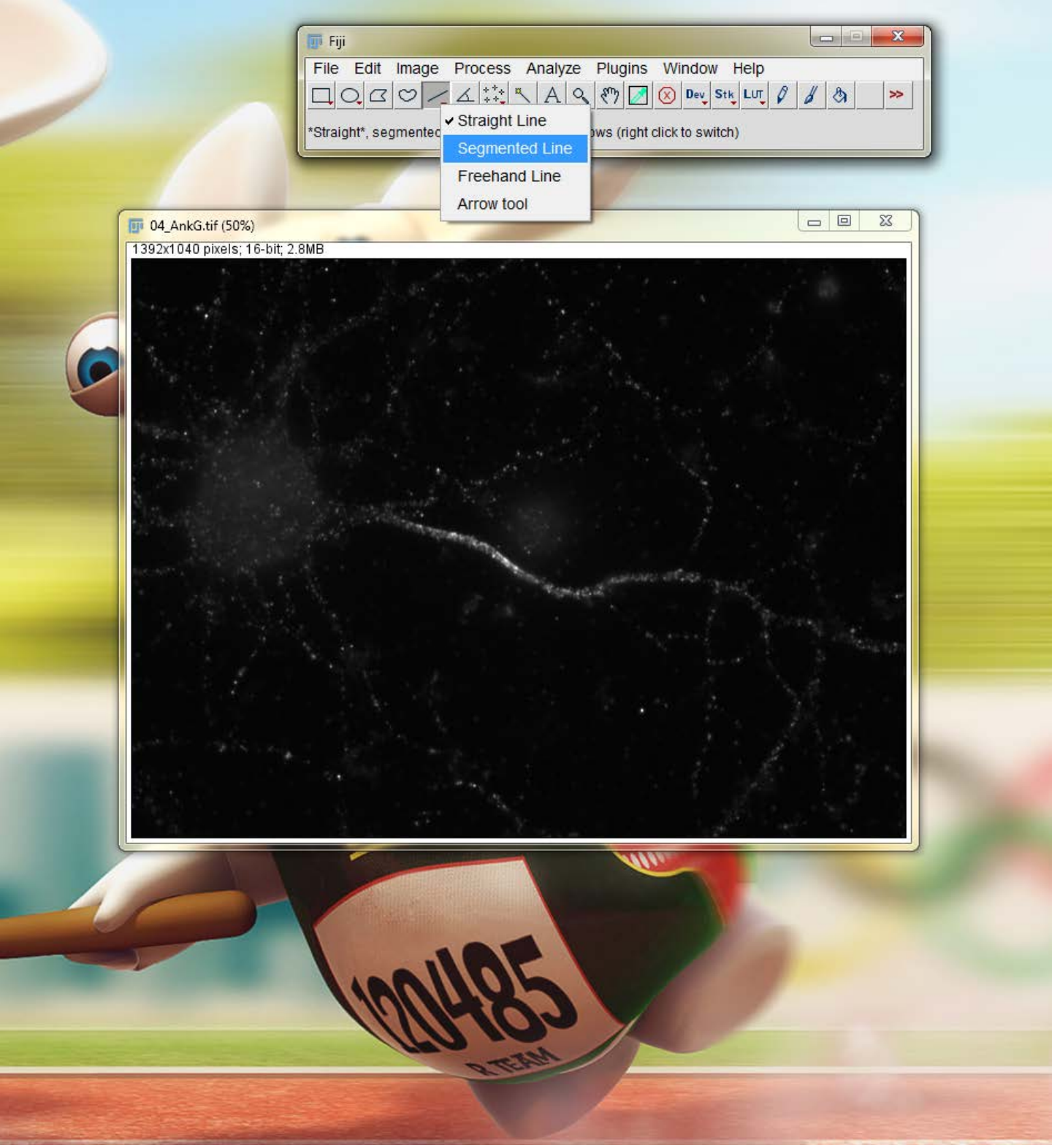
- Is that really the best staining you can get?
- Yes?
- Ok lets continue…



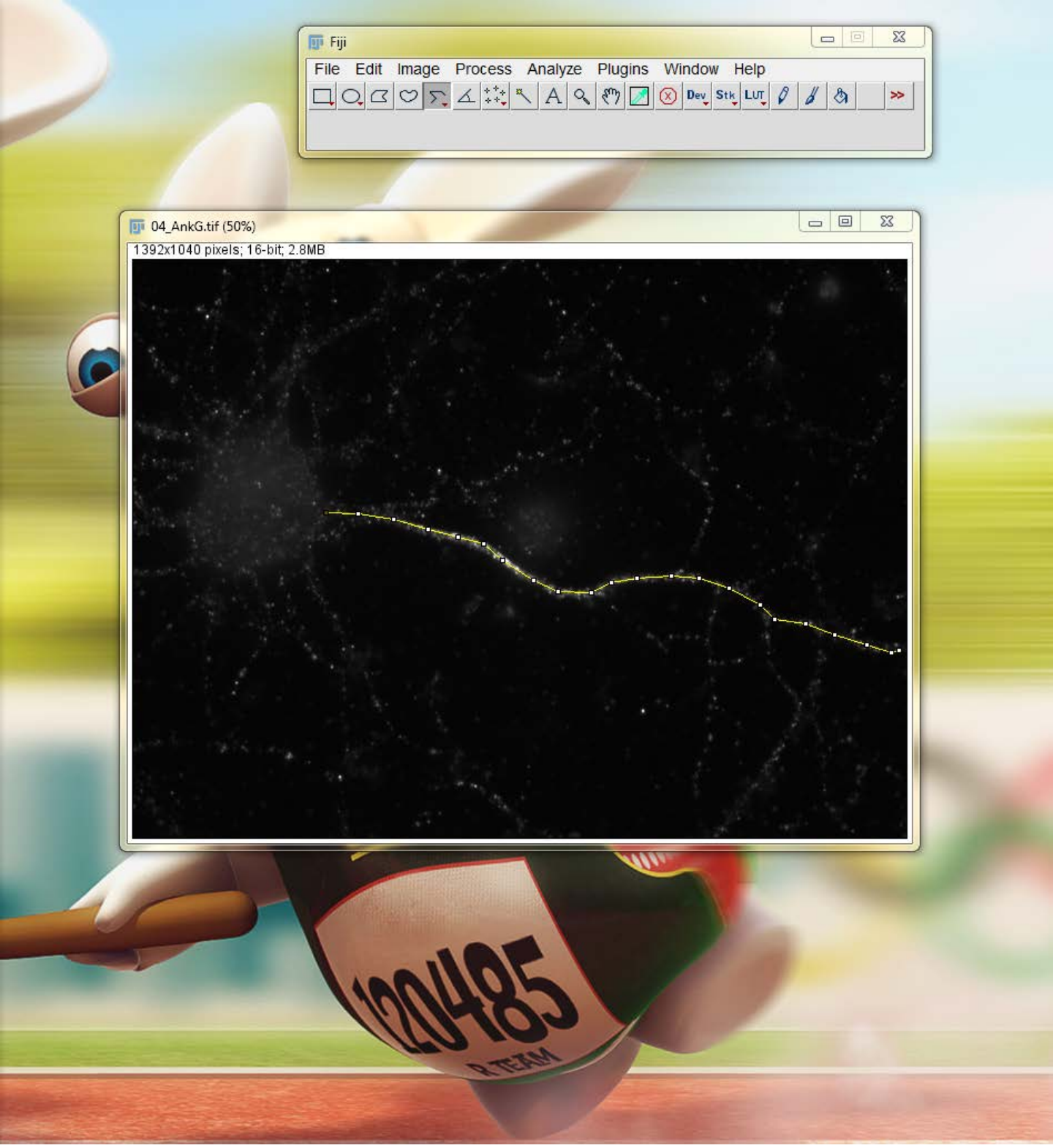
- Check the scale of your picture since some microscope already save the correct scale in the image.



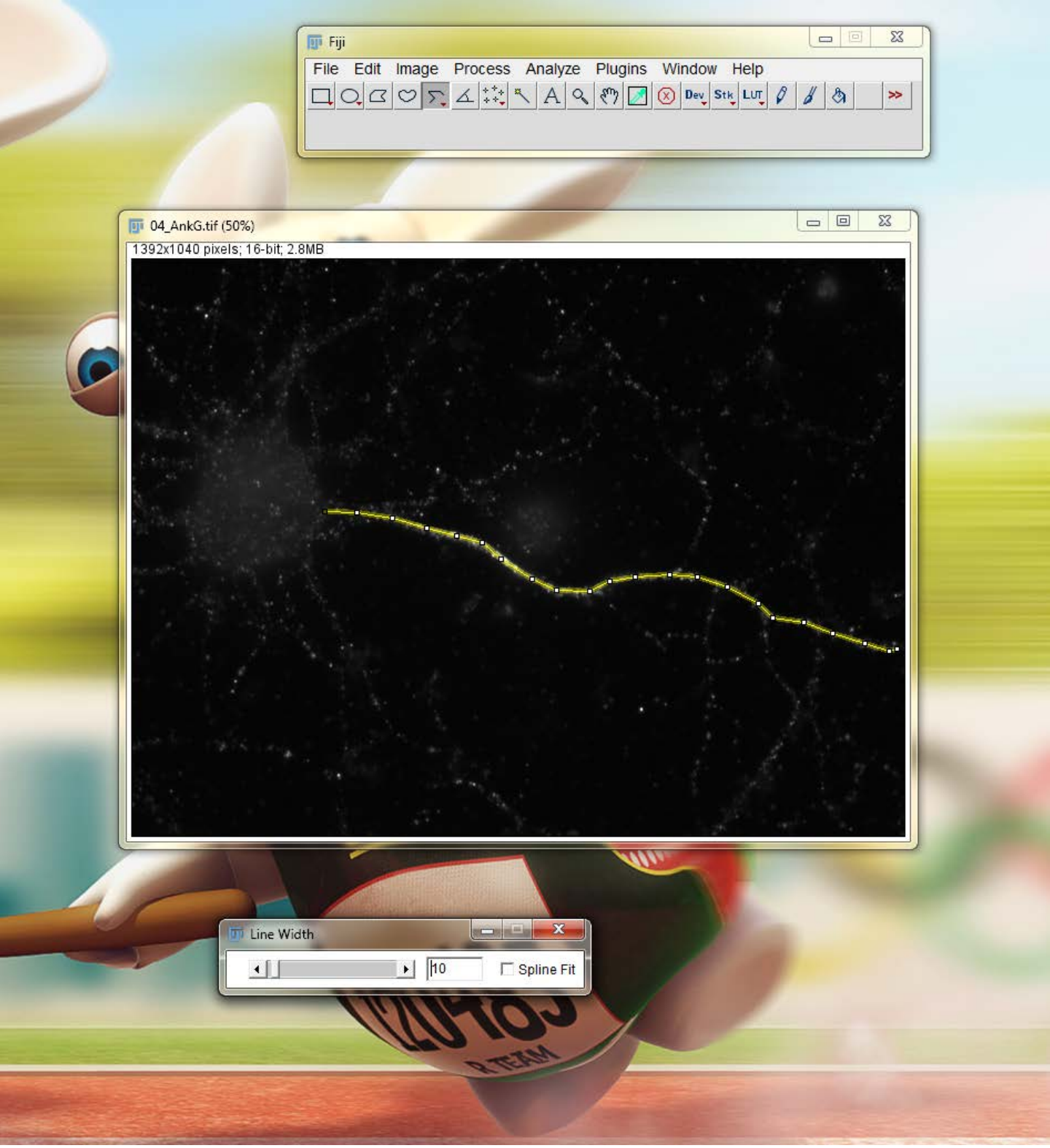
- Shown here is the standard scale of 1 pixel, which is good.
- Sometimes this scale is set to microns per pixel.
- If so, you could either remove the scale by using the button.
- Or you could leave it but then you should keep in mind that you already have the correct scale later on.



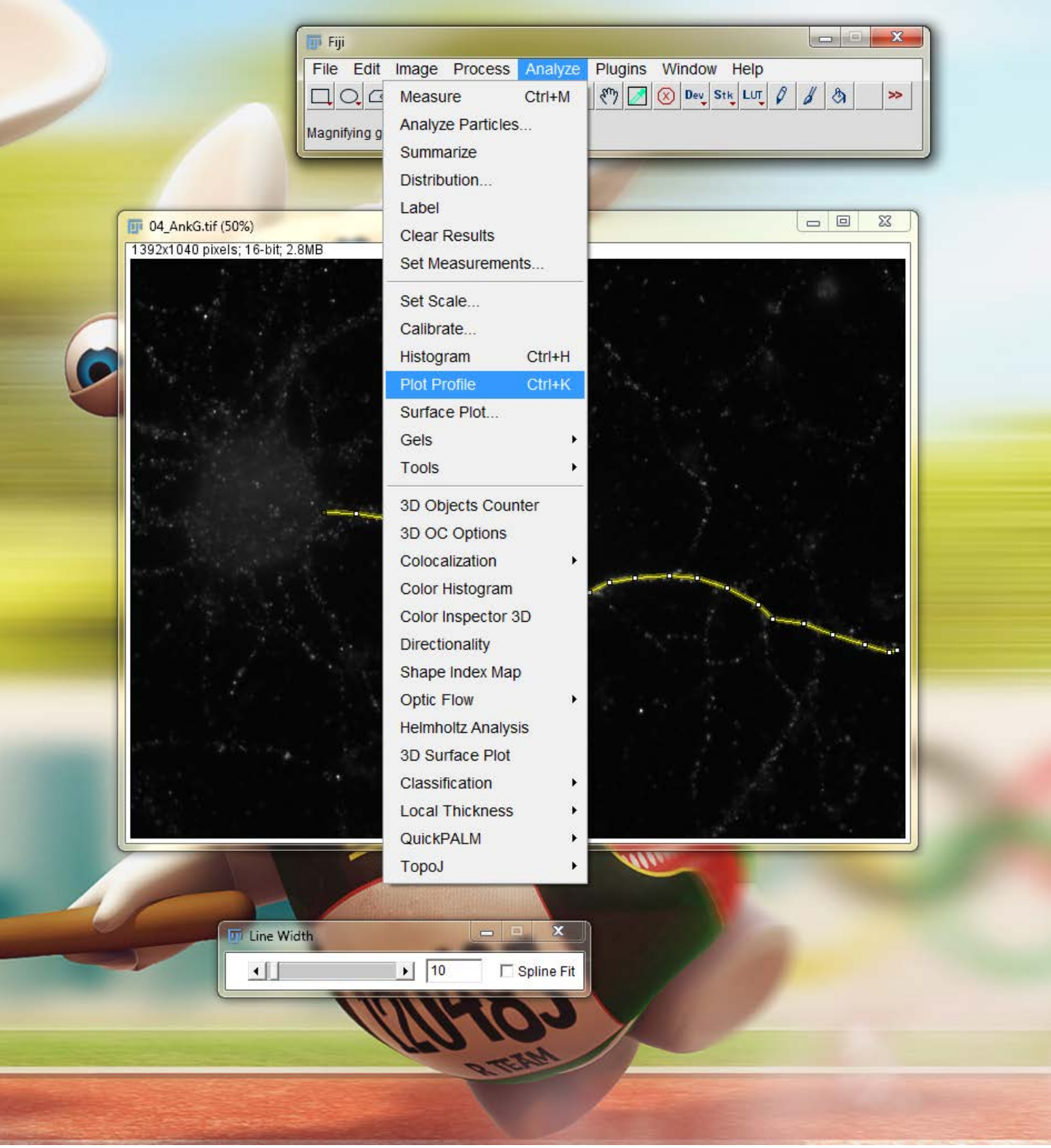
- Open the segmented line tool (right click line tool) and draw a line over the region of interest.
- The region of interest is in this case the axon starting from the soma.


Fiji
File Edit Image Process Analyze Plugins Window Help
Dev Stk LUT
04_AnkG.tif (50%)
1392x1040 pixels; 16-bit; 2.8MB
120485
R TEAM

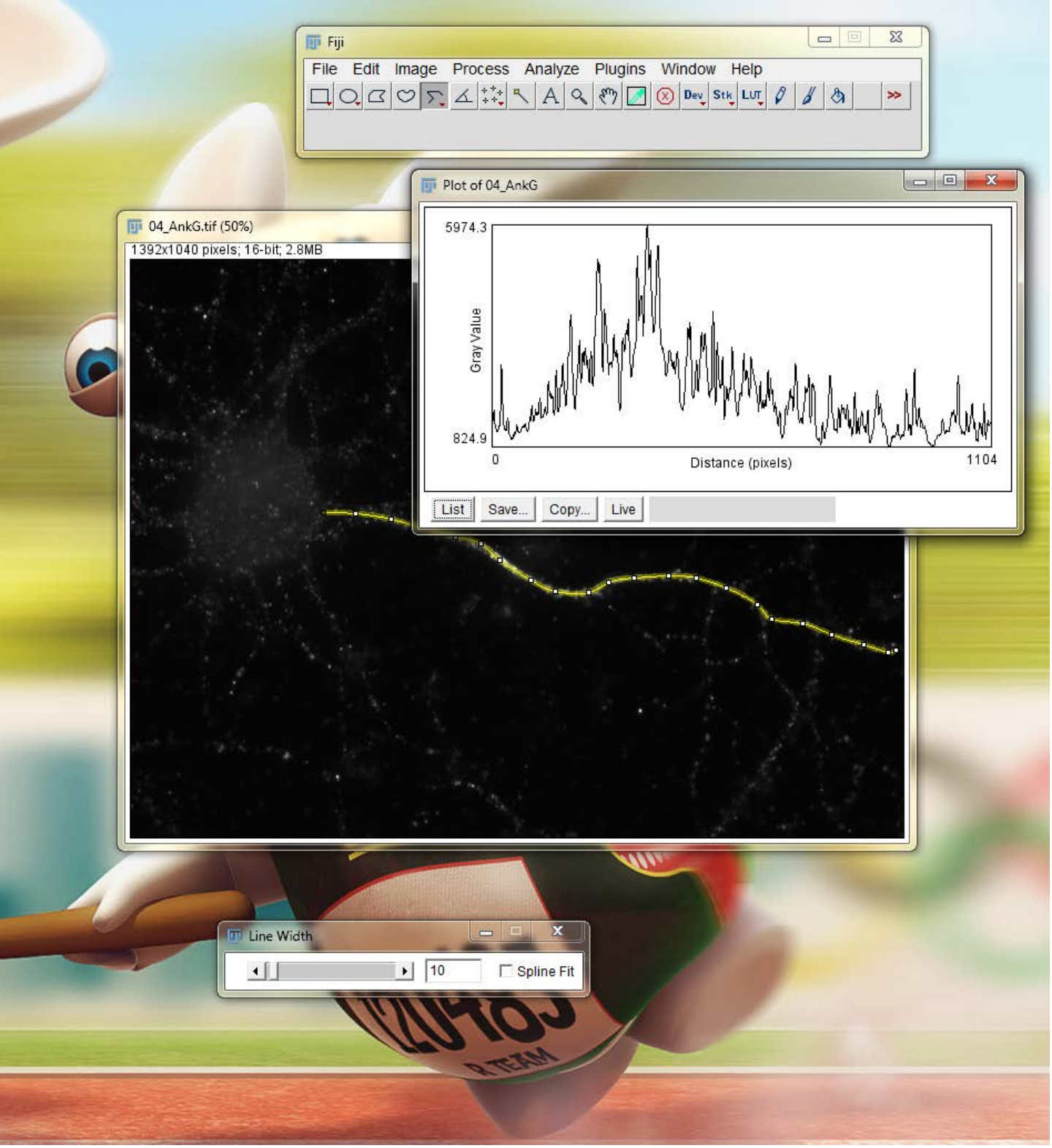
- Like this…



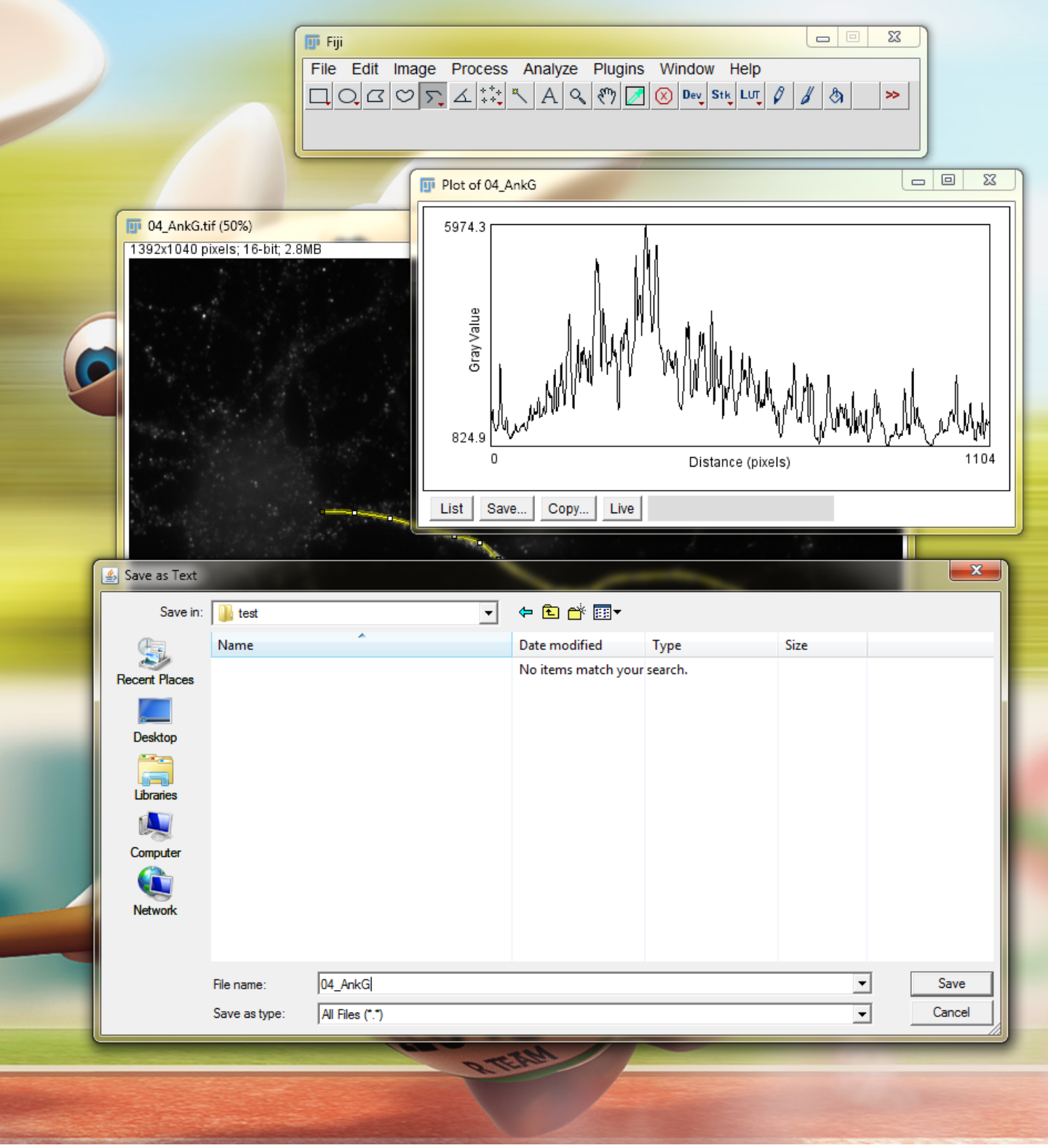
- You can make the line thicker by adjusting the line width (double click the segmented line tool to open line width window)



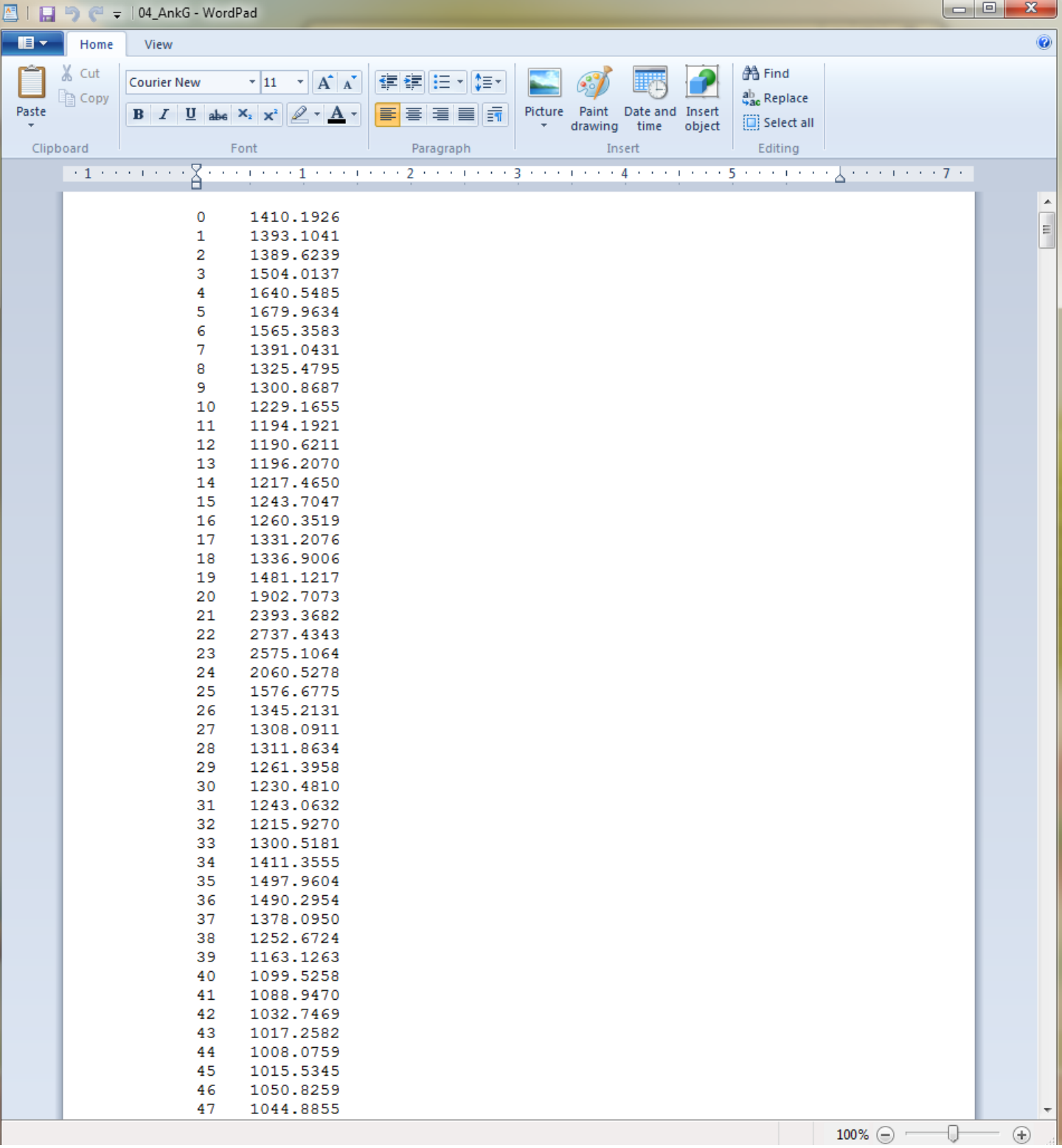
- Plot the profile of the line



- It should look something like this….

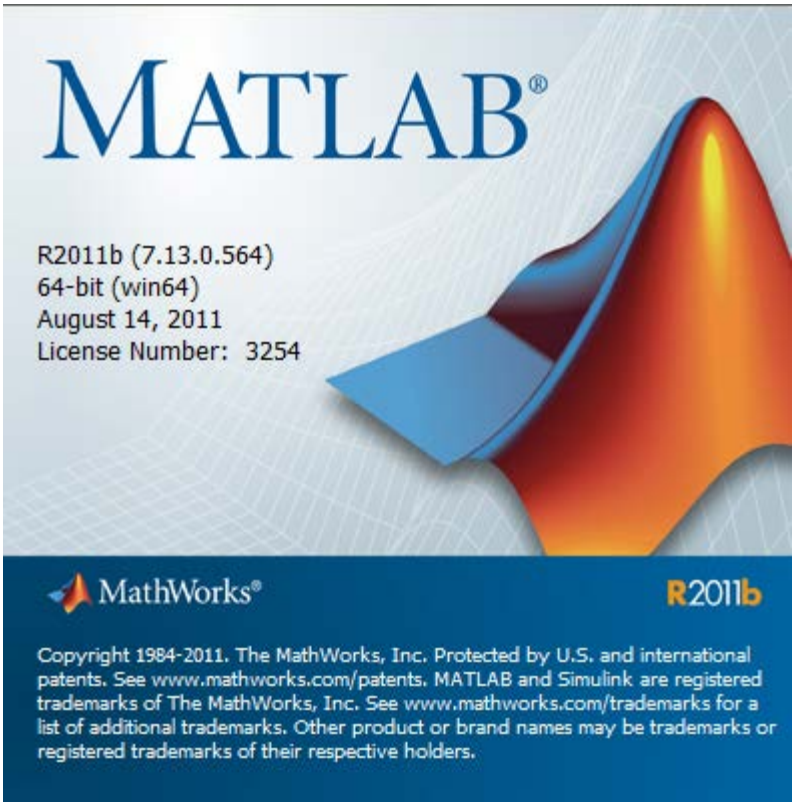


- Click save and save the profile as a .txt file in a folder were you will save all the profiles of a specific staining.
- It is easier the start with a number followed by the staining (this helps to organize your results)

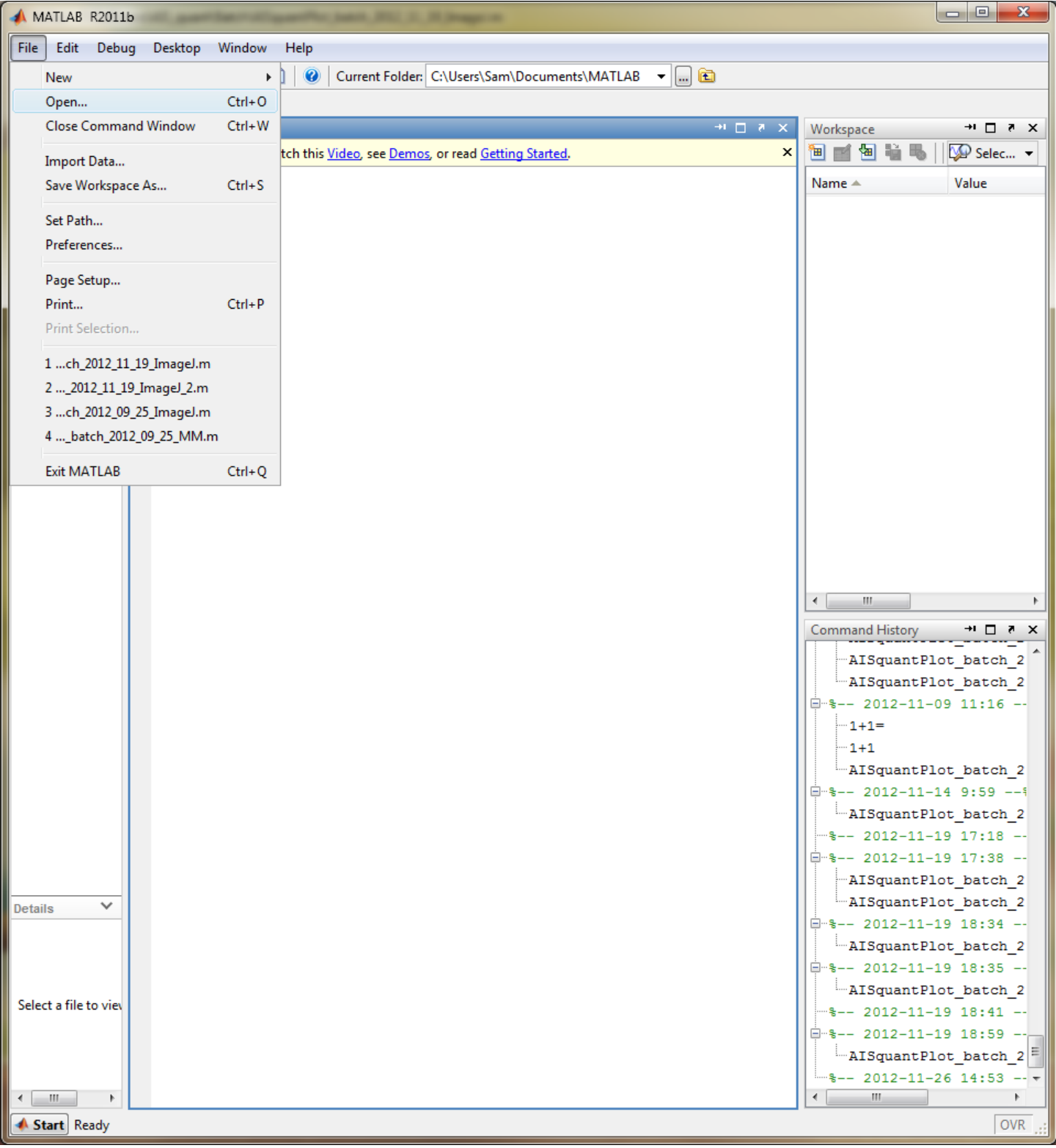


0	1410.1926
1	1393.1041
2	1389.6239
3	1504.0137
4	1640.5485
5	1679.9634
6	1565.3583
7	1391.0431
8	1325.4795
9	1300.8687
10	1229.1655
11	1194.1921
12	1190.6211
13	1196.2070
14	1217.4650
15	1243.7047
16	1260.3519
17	1331.2076
18	1336.9006
19	1481.1217
20	1902.7073
21	2393.3682
22	2737.4343
23	2575.1064
24	2060.5278
25	1576.6775
26	1345.2131
27	1308.0911
28	1311.8634
29	1261.3958
30	1230.4810
31	1243.0632
32	1215.9270
33	1300.5181
34	1411.3555
35	1497.9604
36	1490.2954
37	1378.0950
38	1252.6724
39	1163.1263
40	1099.5258
41	1088.9470
42	1032.7469
43	1017.2582
44	1008.0759
45	1015.5345
46	1050.8259
47	1044.8855

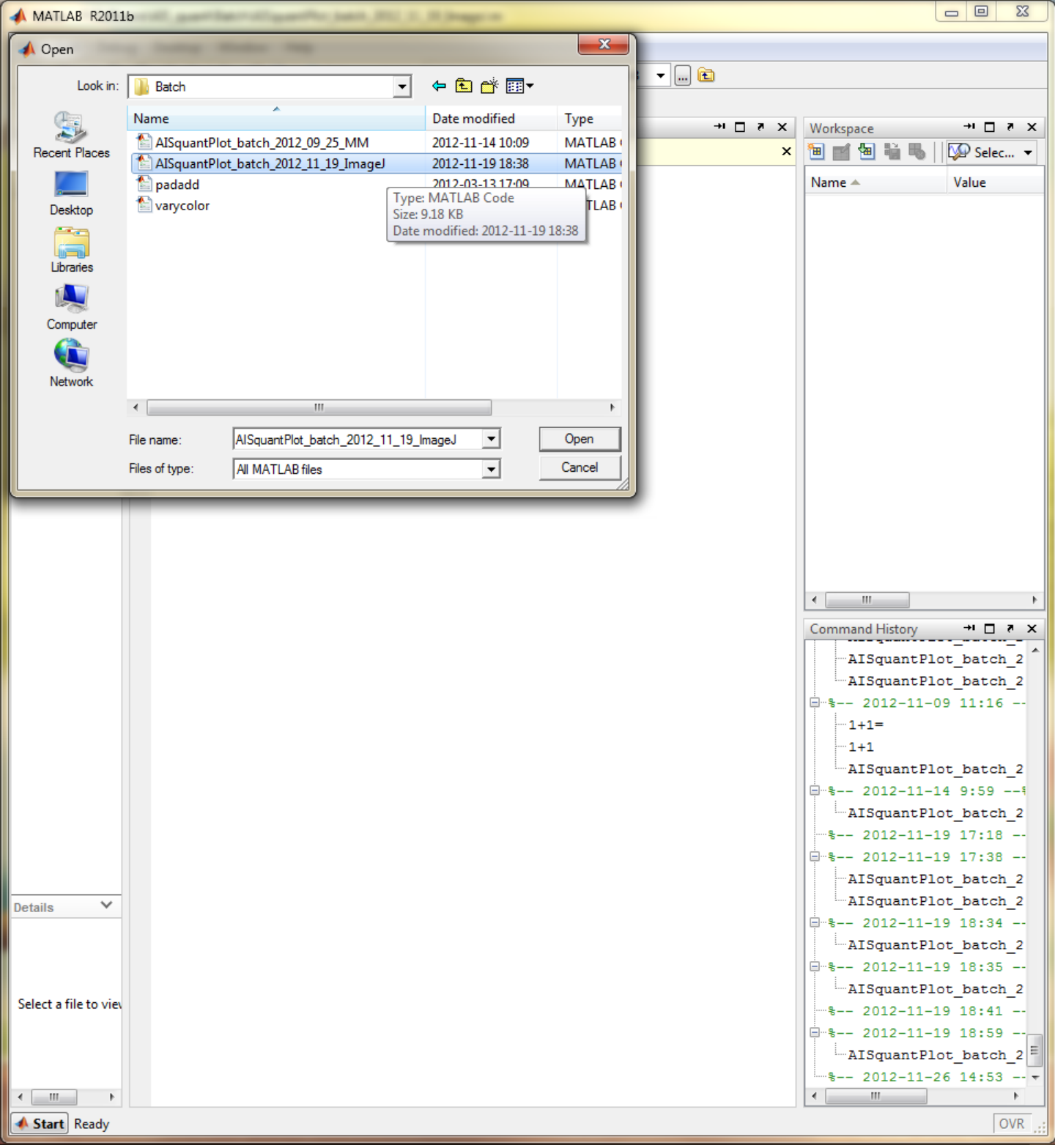
- The .txt file will look something like this…
- In the first column you will find the distance in pixels (or in microns if you did not reset the scale to pixels).
- In the second column you will find the intensity of the line.


MATLAB®
R2011b (7.13.0.564)
64-bit (win64)
August 14, 2011
License Number: 3254
MathWorks®
R2011b
Copyright 1984-2011. The MathWorks, Inc. Protected by U.S. and international patents. See www.mathworks.com/patents. MATLAB and Simulink are registered trademarks of The MathWorks, Inc. See www.mathworks.com/trademarks for a list of additional trademarks. Other product or brand names may be trademarks or registered trademarks of their respective holders.

- Open Matlab



- Open the AIS_quant script



- Be sure that you open the ImageJ version

Editor - F:\Software\AIS_quant\Batch\AISquantPlot_batch_2012_11_19_ImageJ.m

```
clear all;
%%%%%%%%%%%%%%%%%%%%%
% MAIN PARAMETERS
%%%%%%%%%%%%%%%%%%%%%

% INPUT FOLDER should contain .txt files with 3 columns:
% 1)-2) x and y coordinates
% 3) intensity values


%number of averaging points for smoothing.
% bigger values correspond to more smoothing
nAverPoints = 50;
%pixel resolution. number of pixels in one micron.
nPixMkm = 15.8;
%threshold values of normalized intensity to determine
%Start and End values (in range from 0 to 1)
dThreshold = 0.33;
%including tails or not in case of center moving average
bTails = true;

% END OF PARAMETERS
%%%%%%%%%%%%%%%%%%%%%


% open text file with intensity profile
% ask user to locate folder
filesfolder = uigetdir;
%if cancel button is not pressed
if ~isequal(filesfolder, 0)
        disp('Script started.');
        disp('Please wait......');

        %get the list of all files in folder
        dirListing = dir(filesfolder);
        totFiles = length(dirListing);

        %counting all .txt files
        totLogFiles=0;
        for nFile = 1:totFiles
                % checking is it .txt file
                filenamein = fullfile(filesfolder,dirListing(nFile).name);
                fExtension = (filenamein(length(filenamein)-3:length(filenamein)));

                if strcmpi(fExtension,'.txt')
                    totLogFiles = totLogFiles + 1;
                end
        end
        %allocating arrays
        allresults = zeros(totLogFiles, 21);
```

- This is how the script looks like
- There are three important parameters (red box)
- AverPoints: the point that are used for smoothing (I always use the amount of pixels equal to ~ 3µm)
- PixMkm: number of pixels in one micron of the picture (use 1 here when ImageJ already used the correct scale)
- Treshold: treshold value (range 0-1) to determine to start and end values

Editor - F:\Software\AIS_quant\Batch\AISquantPlot_batch_2012_11_19_ImageJ.m

```
clear all;
%%%%%%%%%%%%%%%%%%%%%
% MAIN PARAMETERS
%%%%%%%%%%%%%%%%%%%%%

% INPUT FOLDER should contain .txt files with 3 columns:
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%number of averaging points for smoothing.
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nAverPoints = 50;
%pixel resolution. number of pixels in one micron.
nPixMkm = 15.8;
%threshold values of normalized intensity to determine
%Start and End values (in range from 0 to 1)
dThreshold = 0.33;
%including tails or not in case of center moving average
bTails = true;

% END OF PARAMETERS
%%%%%%%%%%%%%%%%%%%%%


% open text file with intensity profile
% ask user to locate folder
filesfolder = uigetdir;
%if cancel button is not pressed
if ~isequal(filesfolder, 0)
        disp('Script started.');
        disp('Please wait......');

        %get the list of all files in folder
        dirListing = dir(filesfolder);
        totFiles = length(dirListing);

        %counting all .txt files
        totLogFiles=0;
        for nFile = 1:totFiles
                % checking is it .txt file
                filenamein = fullfile(filesfolder,dirListing(nFile).name);
                fExtension = (filenamein(length(filenamein)-3:length(filenamein)));

                if strcmpi(fExtension,'.txt')
                    totLogFiles = totLogFiles + 1;
                end
        end
        %allocating arrays
        allresults = zeros(totLogFiles, 21);
```

- Run the script using the button shown by the red square

Editor - F:\Software\AIS_quant\Batch\AISquantPlot_batch_2012_11_19_ImageJ.m

File Edit Text Go Cell Tools Debug Desktop Window Help

Stack: Base

Run AISquantPlot_batch_2012_11_19_ImageJ.m (F5)

```
clear all;
%%%%%%%%%%%%%%%%%%%%%
% MAIN PARAMETERS
%%%%%%%%%%%%%%%%%%%%%

% INPUT FOLDER should contain .txt files with 3 columns:
% 1)-2) x and y coordinates
% 3) intensity values


%number of averaging points for smoothing.
% bigger values correspond to more smoothing
nAverPoints = 50;
%pixel resolution. number of pixels in one micron.
nPixMkm = 15.8;
%threshold values of normalized intensity to determine
%Start and End values (in range from 0 to 1)
dThreshold = 0.33;
%including tails or
bTails = true;

% END OF PARAMETERS
%%%%%%%%%%%%%%%%%%%%


% open text file wi
% ask user to locat
filesfolder = uiget
%if cancel button is not pressed
if ~isequal(filesfolder, 0)
        disp('Script started.');
        disp('Please wait......');

        %get the list of all files in folder
        dirListing = dir(filesfolder);
        totFiles = length(dirListing);

        %counting all .txt files
        totLogFiles=0;
        for nFile = 1:totFiles
                % checking is it .txt file
                filenamein = fullfile(filesfolder,dirListing(nFile).name);
                fExtension = (filenamein(length(filenamein)-3:length(filenamein)));

                if strcmpi(fExtension,'.txt')
                    totLogFiles = totLogFiles + 1;
                end
        end
        %allocating arrays
        allresults = zeros(totLogFiles, 21);
```

MATLAB Editor

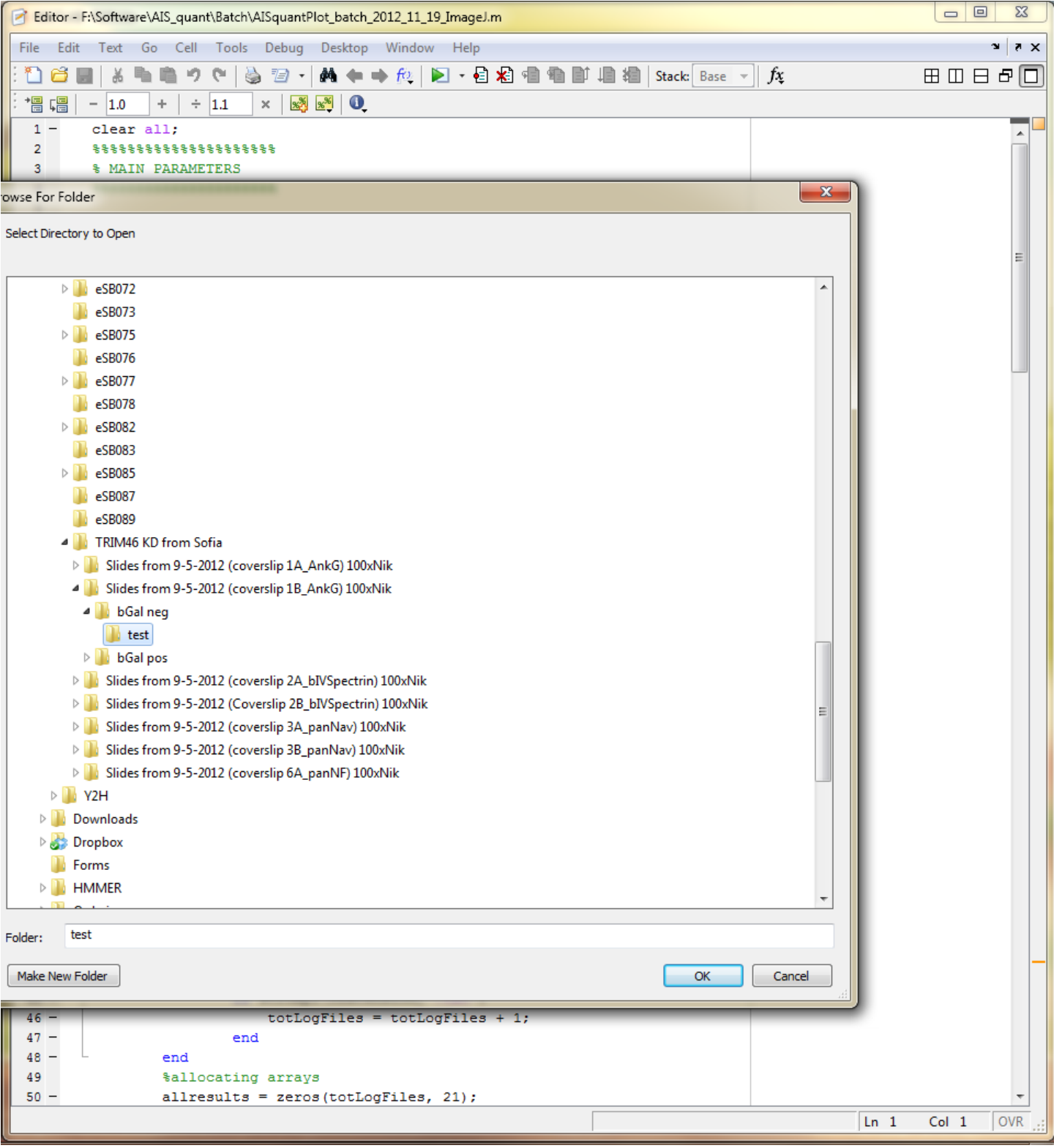
File F:\...\AISquantPlot_batch_2012_11_19_ImageJ.m is not found in the current folder or on the MATLAB path.

To run this file, you can either change the MATLAB current folder or add its folder to the MATLAB path.

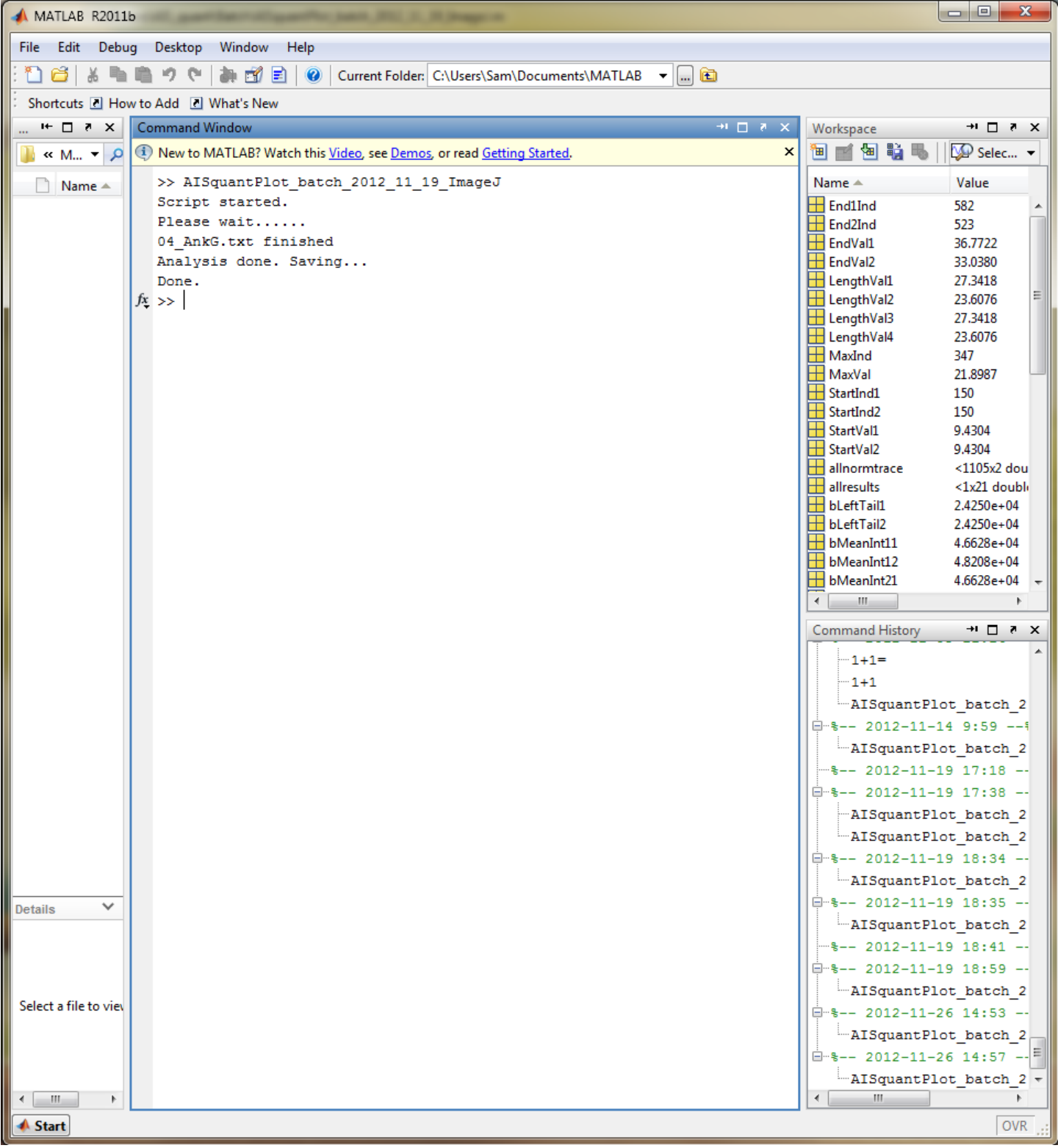
Change Folder | Add to Path | Cancel | Help

Ln 1 Col 1 OVR

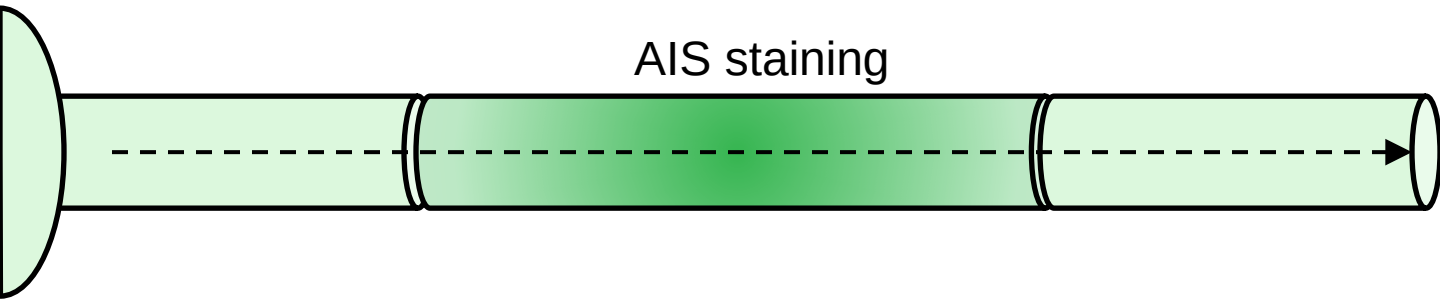
- Click Add to Path

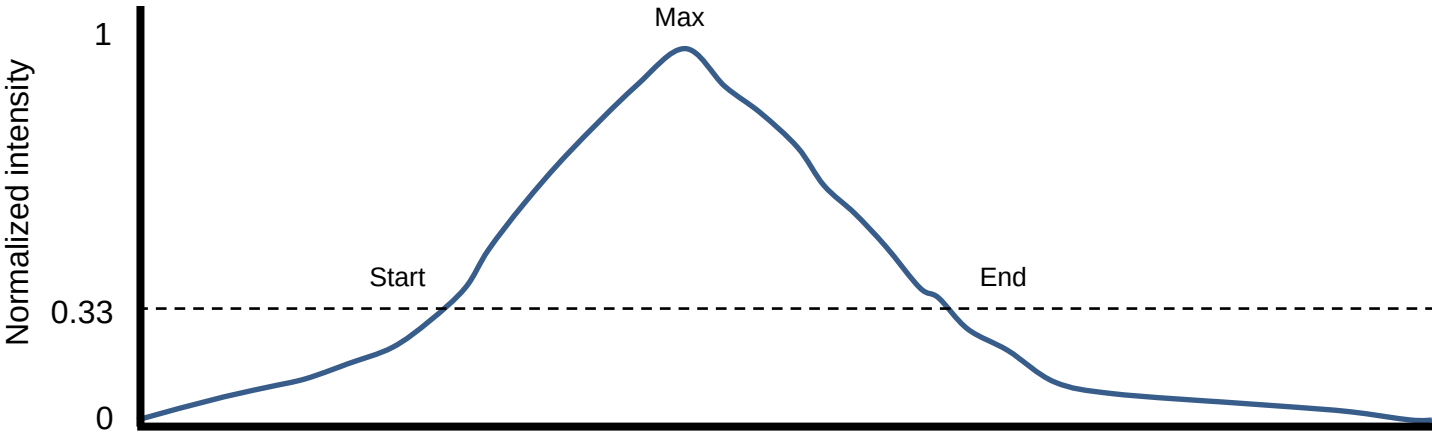


- Locate the folder with your plot data from ImageJ (.txt)
- Click OK



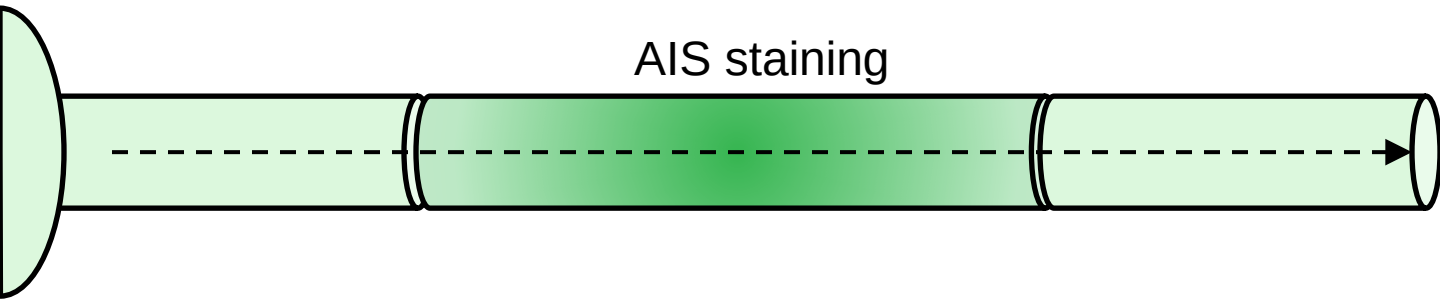
- The script will automatically analyze all .txt files located in the folder and save the results in a single Excel file (named summary)

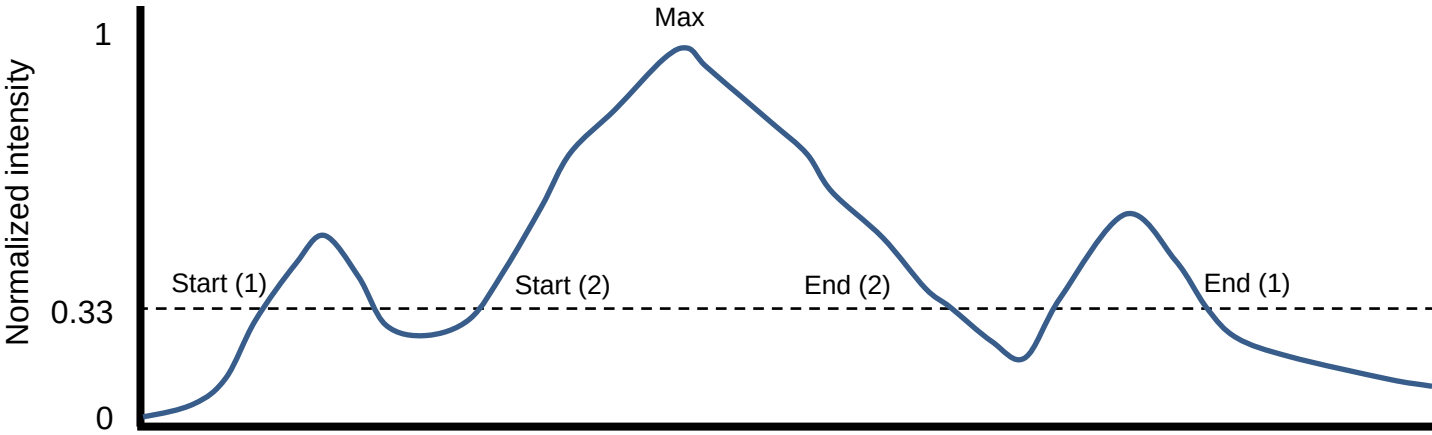




But what was actually analyzed by the script?

- It converts pixels to µm (mkm).
- It automatically normalizes all data from 0 (lowest value) to 1 (highest data).
- It determines the max value of the increased staining, which is equal to the position were the normalized intensity is 1.
- It determines the start and end value of the increased staining, which is equal to the positions where the normalized intensity crosses the threshold.





But what if the threshold is crossed more than twice?

The start is determined by:

- Start(1): from the first data point (increase from the first data point to the threshold)
- Start(2): from the max (decrease from Max to the threshold)

The end is determined by:

- End(1): from the last data point (increase from the last data point to the threshold)
- End(2): from the max (decrease from Max to the threshold)

All start and end values are saved by the script in the Excel file

summary [Compatibility Mode] - Microsoft Excel

File:	04_AnkG.txt
length,mkm	normalized intensity
0	0.135429717
0.063291139	0.133172678
0.126582278	0.130698462
0.189873418	0.128432007
0.253164557	0.125836513
0.316455696	0.123123843
0.379746835	0.120692938
0.443037975	0.118174662
0.506329114	0.116514619
0.569620253	0.115853273
0.632911392	0.115915283
0.696202532	0.115914814
0.759493671	0.11507165
0.82278481	0.113353849
0.886075949	0.111083
0.949367089	0.108480184
1.012658228	0.105929423
1.075949367	0.103124274
1.139240506	0.100346163
1.202531646	0.097633284
1.265822785	0.095084635
1.329113924	0.092858749
1.392405063	0.090690286
1.455696203	0.088755105
1.518987342	0.087214912
1.582278481	0.085628691
1.64556962	0.084301128
1.708860759	0.082983297
1.772151899	0.081970599
1.835443038	0.080559538
1.898734177	0.078714199
1.962025316	0.076820072
2.025316456	0.075145987
2.088607595	0.074113937
2.151898734	0.073227063
2.215189873	0.072600284
2.278481013	0.072402789
2.341772152	0.072243995

In sheet 2 of the Excel file you will find the distance in mkm (µm) and the normalized intensity corresponding to the distance.

summary [Compatibility Mode] - Microsoft Excel

E24

	A	B	C	D	E	F
1	Filename	Start from left (1), mkm	Start from max value(2), mkm	Max, mkm	End from right end (1), mkm	End from max value (2), mkm
2	04_AnkG.txt	9.430379747	9.430379747	21.89873418	36.7721519	33.03797468
3						

summary [Compatibility Mode] - Microsoft Excel

E24

	G	H	I	J	K	L
1	AISlength (End1-Start1), mkm	AISlength (End2-Start1), mkm	AISlength (End1-Start2), mkm	AISlength (End2-Start2), mkm	# of smooth points	intensity thres
2	27.34177215	23.60759494	27.34177215	23.60759494	51	
3						

summary [Compatibility Mode] - Microsoft Excel

D19

	L	M	N	O	P	Q	R
1	intensity threshold	resolution (pixels in mkm)	Tails	Int_Left_tail1, a.u./mkm	Int_Left_tail2, a.u./mkm	Int_Middle_(Start1_End1)	Int_Middle_(Start2_End1)
2	0.33	15.8	1	24250.19154	24250.19154	46627.64844	46627.64844
3							

summary [Compatibility Mode] - Microsoft Excel

D19

	S	T	U	V	W	X	Y	Z	AA
1	Int_Middle_(Start1_End2)	Int_Middle_(Start2_End2)	Int_Right_tail1, a.u./mkm	Int_Right_tail2, a.u./mkm					
2	48208.41151	48208.41151	23524.20319	24853.2043					
3									
4									

In sheet 1 of the Excel file you will find:

- All the different start and end values as well as the max value.
- The length, which is the difference between the different start and end values.
- The parameters you used for the script.
- The mean intensities of the raw dataset between the different start and end values.
- The mean intensities of the raw dataset of the different tails, either before the start or after the end value.

You can now use all this data to determine the position on your staining using, for instance, different conditions.

Are some things unclear?

Try to read this manual again…

Still unclear?

Really?

OK you can always drop by or send me an email with your question….

Cheers,
Sam
(s.vanbeuningen@uu.nl)