

# WIS-NeuroMath

## Neuronal Morphology Analysis Tool

### User Guide

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*WIS-NeuroMath* is based on an efficient multi-scale detection algorithm developed at the Weizmann Institute of Science by Meirav Galun, Ronen Basri and Achi Brandt. The software was written by Meirav Galun, Ofra Golani, Gilad Barkan and Orit Kliper.

Please credit *WIS-NeuroMath* by citing the paper describing its underlying algorithms.

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#### References

M. Galun, R. Basri and A. Brandt, Multiscale edge detection and fiber enhancement using differences of oriented means, ICCV, 2007.

מכון ויצמן למדע  
WEIZMANN INSTITUTE OF SCIENCE



## 1. Index

1. Index .....	2
2. Overview .....	3
3. Installation Instructions.....	5
4. Quick Start .....	6
5. Batch Processing .....	10
6. Neurite Detection.....	12
7. Cell Morphology .....	17
8. Neurite Length Analysis .....	19
9. Ganglion Explant Analysis .....	20
10. Preferences and Settings .....	22
11. Working with high resolution images.....	23
12. Measurement Tools .....	25
13. Miscellaneous.....	27
14. References.....	27

## 2. Overview

WIS-NeuroMath is a software tool for automated quantification of neuronal morphologies in both in vivo and in vitro preparations. WIS-NeuroMath is based on accurate detection of candidate neurites using the algorithm described by Galun et al (2007). It first looks for *edges* in the image and then matches pairs of nearly parallel edges to find *fibers*, which constitute neurite-like structures. The method allows accurate detection of neurites in challenging images. Following neurite detection, three different types of processing can be carried out depending on the desired application:.

### **Cell Morphology of cultured neurons**

Cell bodies are detected using the same image used for neurite detection. It then traces the candidate neurites to allow assignment to the relevant cell bodies. Neurite lengths, branching, cell body area and other parameters are then calculated and saved for each cell, while ignoring irrelevant items (neurites which are not attached to cell bodies, cell bodies that are too small or too big, etc.). Individual cell data are exported to Excel files, as well as image averages. The graphical user interface allows the user to modify detection thresholds and analysis parameters, to perform analyses on single files or whole directories, and to browse through the results.

### **Neurite Length Analysis on Sections**

This mode provides a solution for images in which cell bodies are not present, for example longitudinal nerve sections that contain only neurites and non-neuronal cells. Neurites are detected and connected component analysis is applied to distinguish between neurite elements. A user-set threshold allows exclusion of very short neurite elements if desired, to reduce noise in certain experiments. The number of neurite elements, their average and median length are calculated as well as additional parameters such as the number of branches and the branching complexity.

### **Ganglion Explant Analysis**

Ganglion explant cultures represent a particular challenge since neurites can be very profuse and dense close to the ganglion, and even a human eye cannot distinguish between them. Hence, in this mode the user manually defines an ellipse mask around the ganglion. The software then detects neurites and counts the number of neurites crossing that mask. Neurite numbers can be calculated at several offsets inside and outside the mask, to provide average and median numbers of crossing neurites. The user can set numbers of offset masks and the distance between them as desired; hence this tool bears some resemblance to classical Sholl analyses of dendritic arbors.

### 3. Installation Instructions

WIS-NeuroMath was compiled and tested under Windows XP and Windows 7.

#### System Requirements

Software requirements	PC running Windows XP or Windows 7, either 32 or 64 bit. Matlab Run Time Component (MCR) 7.14 is required (provided at the website).
Current Version	3.4.8
License	<a href="http://www.cs.weizmann.ac.il/~vision/NeuroMath/index.html">http://www.cs.weizmann.ac.il/~vision/NeuroMath/index.html</a>

#### Installation Steps

This section describes how to install *WIS-NeuroMath* on a computer with the Windows XP operating system.

1. Download *WIS-NeuroMath* onto your computer.
2. Double-click on the zip file. Extract all the files into a new folder for example "C:\NeuroMath". Please make sure that there are no spaces in the target path.
3. To run *WIS-NeuroMath* you must have Matlab Component Run Time (MCR) 7.14 installed on your computer. To install it, download the appropriate MCRInstaller for your operating system from the *WIS-NeuroMath* download page, and run it. You should install the 32 bit or 64 bit versions depending on your computer. You must have administrator privileges to do it. MCRInstaller update the system path. In order for this update to work, you should logoff (or restart).
4. To run *WIS-NeuroMath*, double click on either *NeuroMath\_32bit.exe* or *NeuroMath\_64bit.exe* under the *bin* subdirectory (for example click on C:\NeuroMath\bin\NeuroMath\_32bit.exe).
5. The Installation package includes different kinds of sample data, together with Preferences files suitable for those files. The samples files are in the *data* subdirectory while the preferences files are in the *bin* subdirectory.

## 4. Quick Start

This section describes basic usage of *WIS-NeuroMath* with the Cell Morphology module.

1. To run *WIS-NeuroMath*, double click on *NeuroMath\_32bit.exe* (for example click on C:\NeuroMath\bin\NeuroMath\_32bit.exe). The Main window will appear:

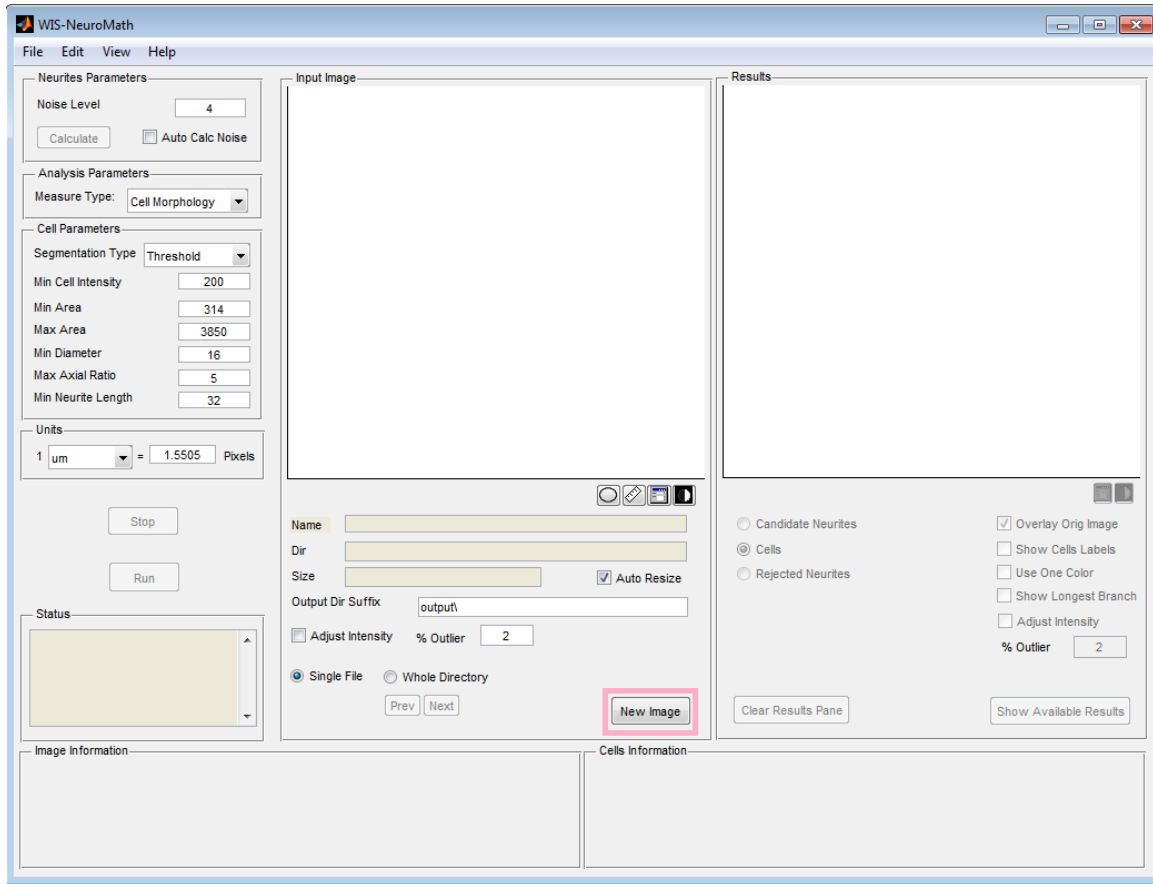


Figure 1: WIS-NeuroMath Main Window

2. To load an image, click the *New Image* button and select the image for analysis in the browse dialog opened. The Image will be shown in the *Input Image* panel. Its file name and path will appear underneath (see Figure 2). The software can handle tif and jpg files.

3. To Process the Image, press the *Run* button.

All buttons and menu items are disabled until processing is done. The status of the process is shown in the *Status* panel on the left side of the window. Processing time depends on the nature of the specific image and on the chosen parameters. In general analysis of images with more neurites takes longer.

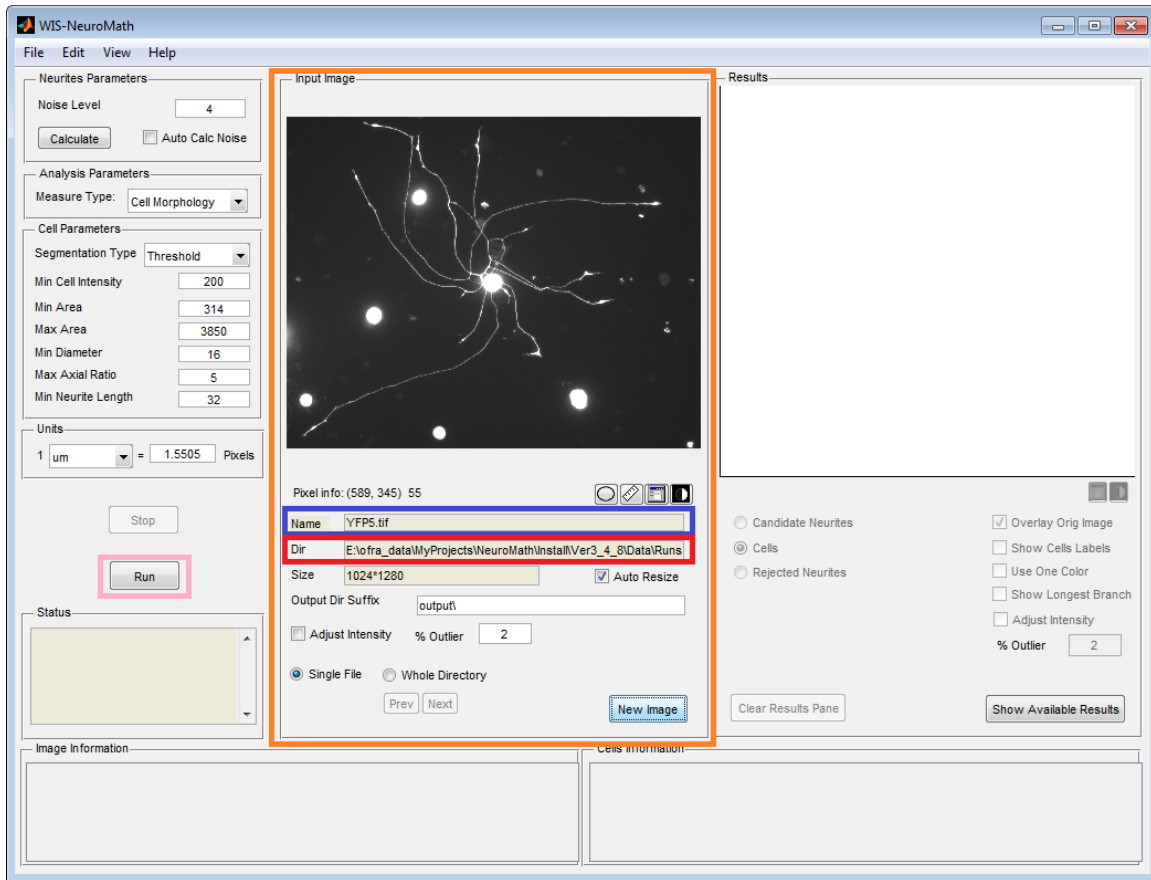



Figure 2: Image Loaded

4. When processing is complete the results are shown visually in the *Results* panel on the right side of the main window. You can toggle between different views of the results, using the radio buttons underneath the Results image (see Figure 3):
  - *Cells*: The detected cell bodies are shown with the neurites attached to them colored with the same color.
  - *Candidate Neurites*: All the candidate neurites found in the first stage are shown.
  - *Rejected Neurites*: Neurites which were not attached to cell bodies are shown. Usually these are neurites whose cell bodies are outside the border of the image. These neurites are not counted in all the cell and overall neurites length measurements.

You can show cell labels by checking the *Show Cell Labels* checkbox.

You can view the result in a separate window using the  button.

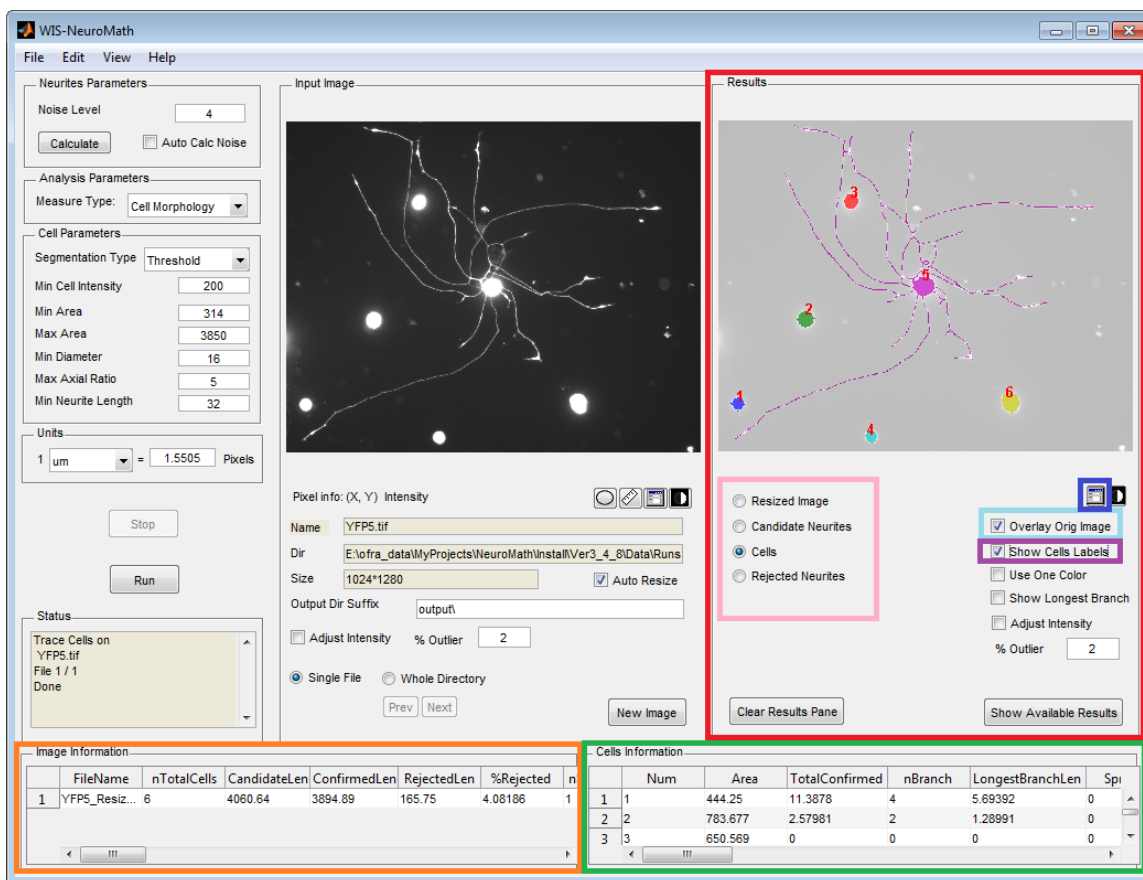


Figure 3: Display results

Overall image measurements are shown in the *Image Information* table on the bottom left side of the window. Table 1 shows the full list of Image measures.

Image Measure Name	Description
nTotalCells	Number of detected cells
CandidateLen	Total length of candidate neurites
ConfirmedLen	Total length of neurites attached to cell bodies
RejectedLen	Total length of neurites not attached to cell bodies.
%Rejected	Percent of neurites not attached to any cell body. Usually these are neurites whose cell body are outside the border of the image. Rarely there are also tracing errors, so watch this value as high value might indicate tracing errors.
nSproutCells	Number of cells for whom the length of the longest neurite exceeds <i>MinNeuriteLength</i> (see figure 4)
%Sprouting	Percent of sprouted cells out of the total detected cells



Image Measure Name	Description
AvgSCLen	Average length of neurites attached to sprouted cells
AvgTCArea	Average area of all detected cell bodies
AvgSCArea	Average area of sprouted cells only
LongestBranch	Length of longest branch in the image
AvgTCIntensity	Average intensity all detected cell bodies
AvgSCIntensity	Average cell body intensity of sprouted cells only
AvgSCNeuriteIntensity	Average neurites intensity of sprouted cells only

Table 1: Image Measures

Per cell measurements are shown in the *Cell Information* table on the bottom right side of the window. Table 2 lists all the cell measures and their description.

Cell Measure Name	Description
Area	Cell area
TotalConfirmed	Total outgrowth, length of neurites attached to the cell
nBranch	Number of main neurites sprouting from the cell body.
LongestBranchLen	Length of the cell's longest branch
Sprout?	A flag indicating whether a cell is sprouted (1) or not (0)
Cross?	A flag indicating whether the neurites of this cell cross with those of another cell. In that case the tracing algorithm does not try to solve the ambiguity, but splits the neurite between the two cells at the point of equal distance from the two cell bodies. Thus in cases where there is a cross the total confirmed length might not be "correct" and the average values might be more suitable.
CellIntensity	Average intensity of the cell body, this can be used to measure protein expression level
NeuriteIntensity	Average intensity of the cell's neurites, this can be used to measure protein expression level. This is an approximation to the neurites' intensity that is based on the "skeletonized" neurites instead of the full width neurites.
MajorAxis	Length of the major axis of the ellipse that has the same normalized second central moments as the region
MinorAxis	Length of the minor axis of the ellipse that has the same normalized second central moments as the region
AxialRatio	Major Axis / Minor Axis
AvgLongestLength	Each neurite pixel is assigned to specific branch (process). Thus we can measure the total outgrowth and longest process of each specific branch (Proc Len).

Cell Measure Name	Description
	AvgLongestLength is the average length over all longest processes.
MedianLongestLength	Median length over all longest processes.
MaxLongestLength	Maximum over all longest processes.
MeanProcLen	Average length over all processes.
MedianProcLen	Median length over all processes.
MaxProcLen	Maximal length over all processes.

Table 2: Cell Measures

All the measurements are in the units (or squared units for area) defined in the Units panel (see Figure 4). In this panel the user should specify the scaling factor from image pixels to microns, which depends on the resolution and magnification used during the acquisition. The software converts pixels to microns automatically and provides measurements in microns.

- Results are saved into two files: *Averaged Results File* includes one line for each Image with the content of *Image Information* table, and an additional line with average over all images (see batch processing). The *Detailed Results File* includes one line for each detected cell, in each processed image, with the content of *Cell Information* table. The files are placed under *Dir\Output Suffix Dir\File Name*. Their default names are set using Edit->Settings. They can be saved either in Excel format (.xls) or comma separated text file (.txt), and can be opened with Excel for example.
- To exit *WIS-NeuroMath*, click on the *File* menu on the upper right side of the window, and click the *Exit* item, or click the x on the upper right side of the window.

**Tip:** If you want to check the effect of changing parameters, you can use different output file for each parameter set (*Output Suffix Dir*), and use *Show Available Results* button to check the results.

## 5. Batch Processing

*WIS-NeuroMath* provides two modes of operation: *Single Image* and *Whole Directory*. You switch between the modes using the radio buttons in the *Input Image* panel (see Figure 4). The modes differ in the following manner:

- Loading files: A file browser that let you select an image file is opened for *Single Image* mode. A directory browser that let you select a directory is opened in the *Whole Directory* mode. A list of all image files in the directory is created internally for further use.

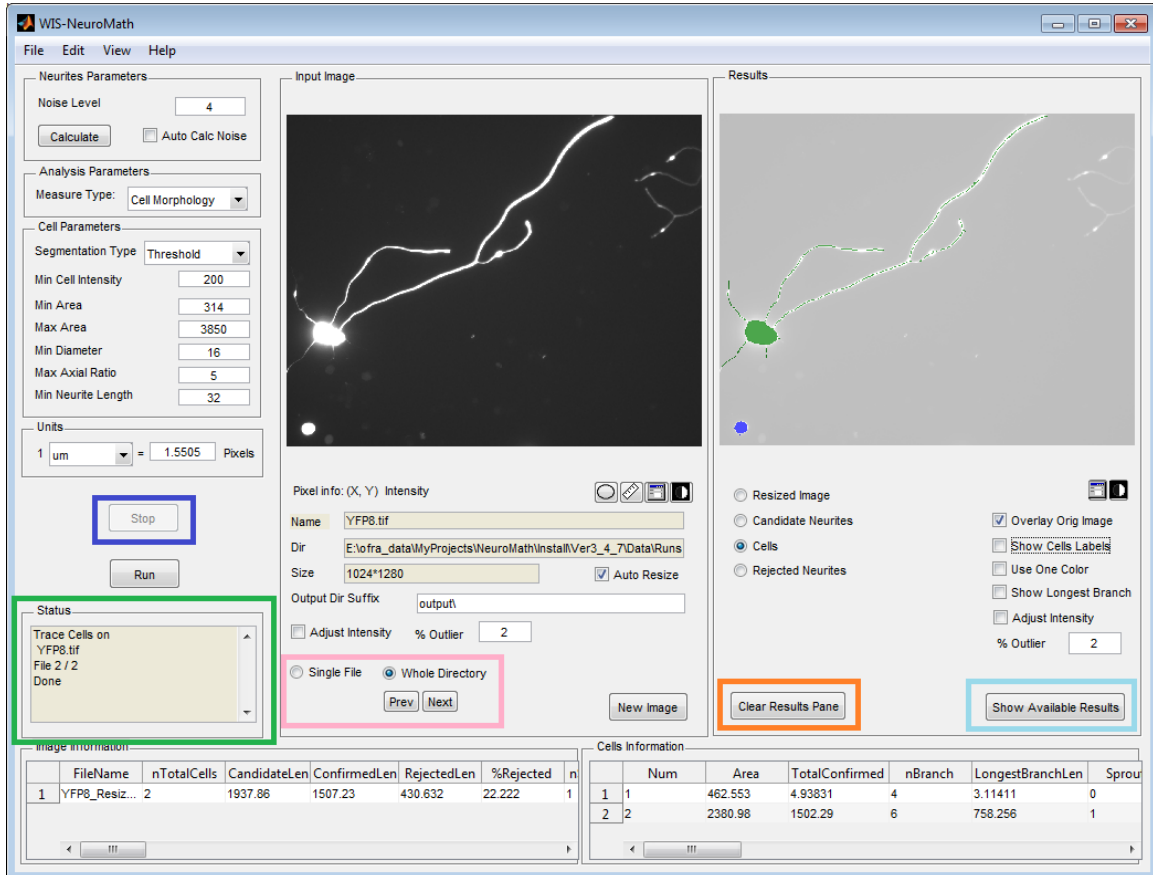


Figure 4: Batch Processing

- In *Whole Directory* mode you can browse through the images in the directory using the *Prev/Next* buttons. These buttons are disabled in *Single Image* mode.
- You can view results of previous runs by clicking the *Show Available Results* button. A message box will appear if no results are available. Just click *OK* to close it (and click *Run* if you want to process the image). If you click *Show Available Results* in *Whole Directory* mode, then browsing the images using the arrow buttons will browse through the results as well. If results are not available for an image a message box will appear. If you want to browse only the original

- images and not the results click the *Clear Results Pane* button. This will clear only the display and will not erase any existing results.
- *Run*: in Whole Directory mode clicking the Run button, will process all the images in the directory. This is very useful for processing many files. You can browse through the images using *Whole Directory* mode, and switch to *Single Image* when you find the image you were looking for, to process only this image.
  - *Stop*: in Whole Directory mode you can stop the batch processing of images by clicking the *Stop* button. The processing will not stop immediately but when processing of the current image is done.

The results files are replaced each time you click the Run button, however, per image temporary files of images which are not involved in the current Run are not removed.

## 6. Neurite Detection

Neurite detection is the basis for all the quantification done by *WIS-NeuroMath*.

Neurites detection is done using an algorithm which first looks for *Edges* in the image and then matches pairs of nearly parallel edges to find *Fibers*, which constitute the neurites. It was developed using florescent images in which the neurites are bright on a dark background. However, one can choose to look for dark fibers on a bright background.

The following section describes the role of the different parameters in the above process. The tool enables you to adjust them according to your image requirements. A detailed description may be found in the references listed. Note that for getting started the default parameters or those in the provided preferences files (see Preferences and Settings below) are usually sufficient and no changes are necessary for most of them.

Some neurite detection parameters are set through the main window, while most of them are set through the Advanced Parameters window.

During Parameter tuning, you can work only on neurite detection without quantification by setting the Measure Type to *None* (see Figure 5). When you are satisfied with the neurites detection, you can use the current neurites and tune the quantification parameters

only by selecting the proper *Measure Type* in the main window setting *Edge/Fibers* to *None* in the Advanced Parameters window (see Figure 6). You should remember to set it back to *fibers* when you want to run it from the beginning.

## Main Window

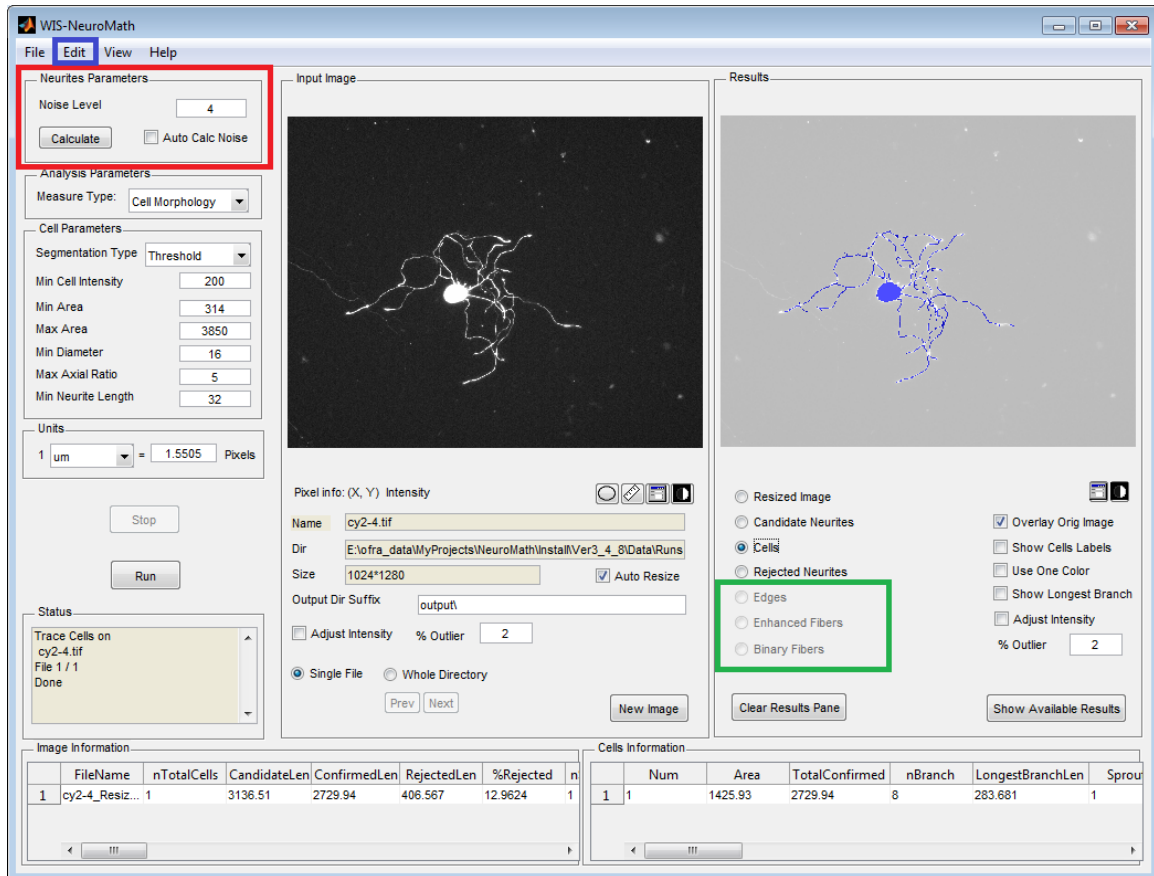


Figure 5: Setting Neurite Parameter

Sensitivity of neurite detection is controlled by the *Noise Level* parameter. *Noise Level* can be fixed for all the images or different for each image.

*Noise Level* is a threshold that controls the edge detection stage of the algorithm. It's typical values should be in the range of 2-6 for 8 bit florescent images. You should consider changing this value in cases such as:

- The image contains low intensity neurites which are not recognized by the default parameters. Try lowering the value to ~2.

- The candidate neurites results include many small isolated parts ("noise"). Try using higher values (5-6).

You can calculate the Noise level of the image using the *Calculate* button. This calculation is controlled by the *Noise Level Percentile* parameter in the Advanced Parameters Window (reasonable values are at 0.7-0.9 range).

You can choose to work with a fixed threshold for all the images or with automatically calculated threshold specific to each image by checking the *Auto Calc Noise* checkbox.

Please note that in general analysis with lower values takes longer.

### **Advanced Parameters Window**

To edit advanced parameters, click the *Edit* menu (see Figure 5) and then the *Advanced Parameters* item. The Advanced Parameters window will appear (see Figure 6).

This set of parameters is used to control advanced behavior of the underlying algorithms. Description of some parameters in this window is beyond the scope of this User Guide.

Neurite Detection is done in the following steps:

- Multi scale edge detection
- Matching parallel edges to form *Enhanced Fibers* picture
- Setting a threshold on the *Enhanced Fibers* to select the whole neurites (*Binary Fibers*).
- Thinning the binary fibers to get their skeleton (1-pixel width lines), which are the candidate Neurites.

Usually only the final candidate neurites picture is saved. You can see intermediate results by selecting the *Save Edges Picture*, *Save Enhanced Fibers Picture* and *Save Binary Files Picture*. This may be useful while tuning parameters, but should be set off when running on many files as it take considerable disk space.

### Edge Detection Parameters

Sensitivity of the Edge Detection is controlled by *Noise Level* that was described above and by the following other parameters:

*Mask Width* – defines the width of the mask around the edge that is used for edge detection. A mask of 3 pixels compares the intensity values of 1 pixel from each side of the examined pixel. A mask of 5 pixels compares the intensity values of 2 pixels from each side of tested pixel, while a mask of 7 compares 3 pixels from each side of tested pixel. Usually a mask of 3 pixels is sufficient, use wider masks for noisier images.

Edge detection is done using a multi-scale algorithm which builds long straight edges from shorter ones. Up to 7 levels ( $2^6 = 64$  pixels in straight lines) can be used but usually for biological data where the neurites are curved the 4 (8 pixels) or 5 (16 pixels) levels are sufficient. *Max Level* controls the number of levels used.

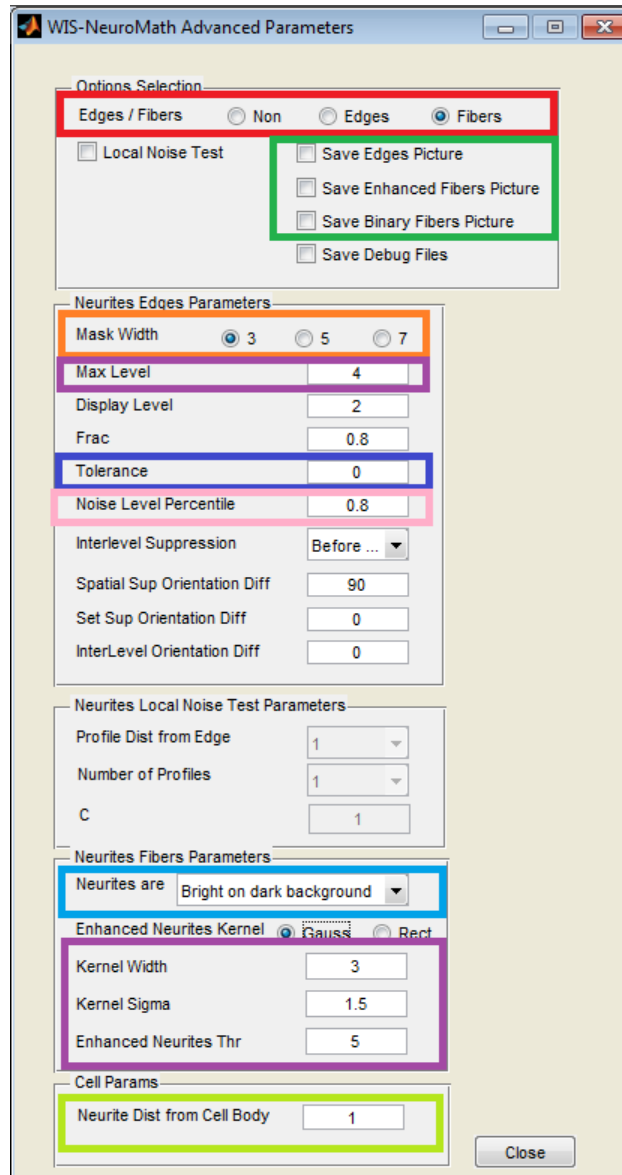


Figure 6: Advanced Parameters Window

The *Tolerance* parameter let you accept broken edges. Its value is between 0-1, and it defines the length of maximal allowed percent of gaps within an edge. A value of 0 does not allow gaps, while a value of 0.375 allows a gap of up to 3/8 of the edge.

### Fiber Detection Parameters

When working with florescent images neurites are *bright on a dark background*. For other application you may look for fibers which are *dark on a bright background*.



Fibers are detected by matching parallel edges using a kernel of width *Kernel Width* and height of *Kernel Sigma*. These two parameters control the distance between the parallel edges (in pixel units for *Kernel Width*). Use higher values of *Kernel Width* to allow detection of wider neurites. However this should be done with caution as selecting too big *Kernel Width* may cause two close neurites to be detected as one neurite.

*Enhanced Neurites Thr* is the threshold used for conversion between the enhanced Fibers picture to the Binary picture. Lower values enable detection of harder to find Neurites. Note that choosing too low values might result in "Noisy" results.

## 7. Cell Morphology

The same image serves to identify both neurites and neuronal cell bodies, the latter detected using simple threshold based segmentation

The following parameters control the Cell Morphology module:

*Min Cell Intensity*: A minimum threshold value for cell body intensity. This value is used for cell bodies detection. Only areas with higher intensity are considered as cell body candidates. Thus unfocused cells and non-florescent cells are ignored. You can use *Pixel Info* for measuring cell intensity (see chapter 12).

*Min Area*: is a minimum threshold value for cell body area. Smaller cell bodies are ignored during the analysis. This is used in order to discard small particles which are not really cell bodies.

*Max Area* is a maximum threshold value for cell body area. Larger cell bodies are ignored during the analysis. This is used in order to reject cell aggregates during the analysis of the image.

*Min Diameter* is a minimum threshold value for cell body diameter (assuming the cell is round). This is another mean (in addition to *MinArea*) to reject small cell-body-like particles. You can use the *Distance tool* for measuring cell diameter (see chapter 12).

*Min Neurites Length* controls the "sprouting" decision. Cell bodies whose longest neurite is longer than this value are declared "sprouted" for further overall averaging. Cells with

lower values are declared "non-Sprouted" and are not taken into account for calculating "Average Sprout Cell Area", and "Average Neurites Length Per Sprout Cells".

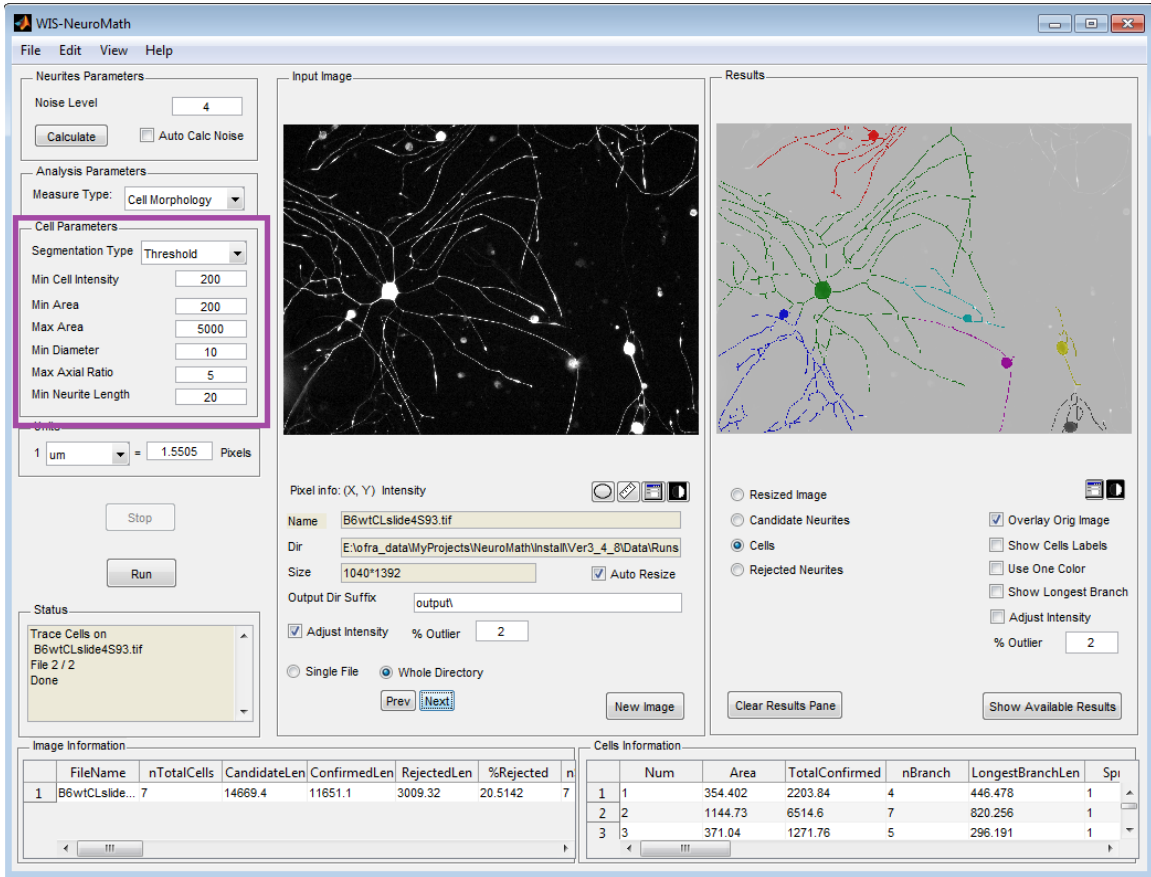


Figure 7: Cell Morphology Parameters

*Neurite Dist from Cell Body* (from the Advanced Parameters window) controls the process of attaching neurites to cell bodies. It defines the maximal distance (in pixels) of the neurite from the cell body. This is done in order to overcome short gaps that cause neurites not to be attached to the cell body. You might want to enlarge its value if you have neurites which were not attached to their cell body.

*Measurement Unit*: Can be either Pixel, mm or um (the default). This is the measurement units used for all results reporting, and the units of the cell body parameters.

*Unit to Pixel conversion*: Number of pixels for each measurement unit. Set this value according to your microscope & experimental setup.

## 8. Neurite Length Analysis

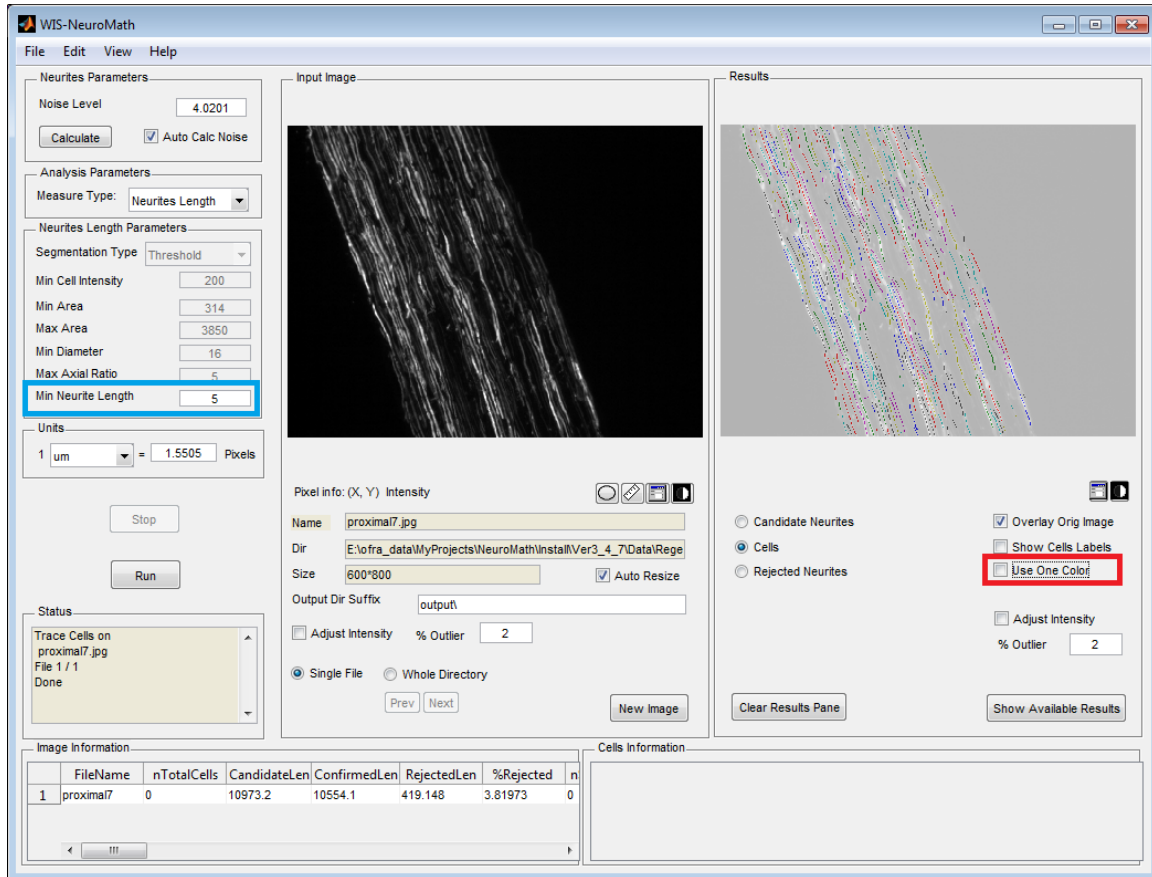


Figure 8: Neurite Length Parameters

This module provides a solution for images in which cell bodies are not present, for example longitudinal nerve sections that contain only neurites and non-neuronal cells. Neurites are detected and connected component analysis is applied to distinguish between neurite elements. *Min Neurite Length* is a threshold which allows exclusion of very short neurite elements if desired, to reduce noise detection in certain experiments.

By default each connected component is shown in different color. You can show all the neurites in one color by checking the *Use One Color* checkbox.

The following table shows the specific measures of this module. They are shown in the *Image Information Table*.



Parameter Name	Description
nFiberElements	Number of distinct neurite elements
AvgFiberElementLength	Average length of neurite elements
MedianFiberElementLength	Median length of neurite elements (50 <sup>th</sup> percentile)
Prctl70FiberElementLength	70 <sup>th</sup> percentile of neurite elements' length
Prctl80FiberElementLength	80 <sup>th</sup> percentile of neurite elements' length
Prctl90FiberElementLength	90 <sup>th</sup> percentile of neurite elements' length
nTotalBranchPoints	Number of branching points in all candidate neurites
nConfirmedBranchPoints	Number of branching points in confirmed neurites
BranchingComplexity	number of confirmed branches / confirmed neurite length

Table 3: Neurite Length Measures

## 9. Ganglion Explant Analysis

### Neurite Count

In this mode the user manually defines an ellipse mask around the ganglion, the software detects neurites and counts the number of neurites crossing that mask. The software can count neurite numbers at several offsets inside and outside the mask, to provide average and median numbers of crossing neurites. The user can set numbers of offset masks and the distance between them as desired using the *Number of Offsets* and *In/Out Masks Offset* fields (see Figure 9).

Use the  button to define a mask. You can do this either in the main window or in a separate window (use the  button). To save the mask click the mouse' right button. A context menu will be shown which let you delete or save the mask. The masks are saved into *Masks Sub Directory*, the name of the mask is set the file name with extension defined by *Mask File Suffix*. These values can be set using the Settings window (see Figure 10), which let you also set other default properties of the Mask.

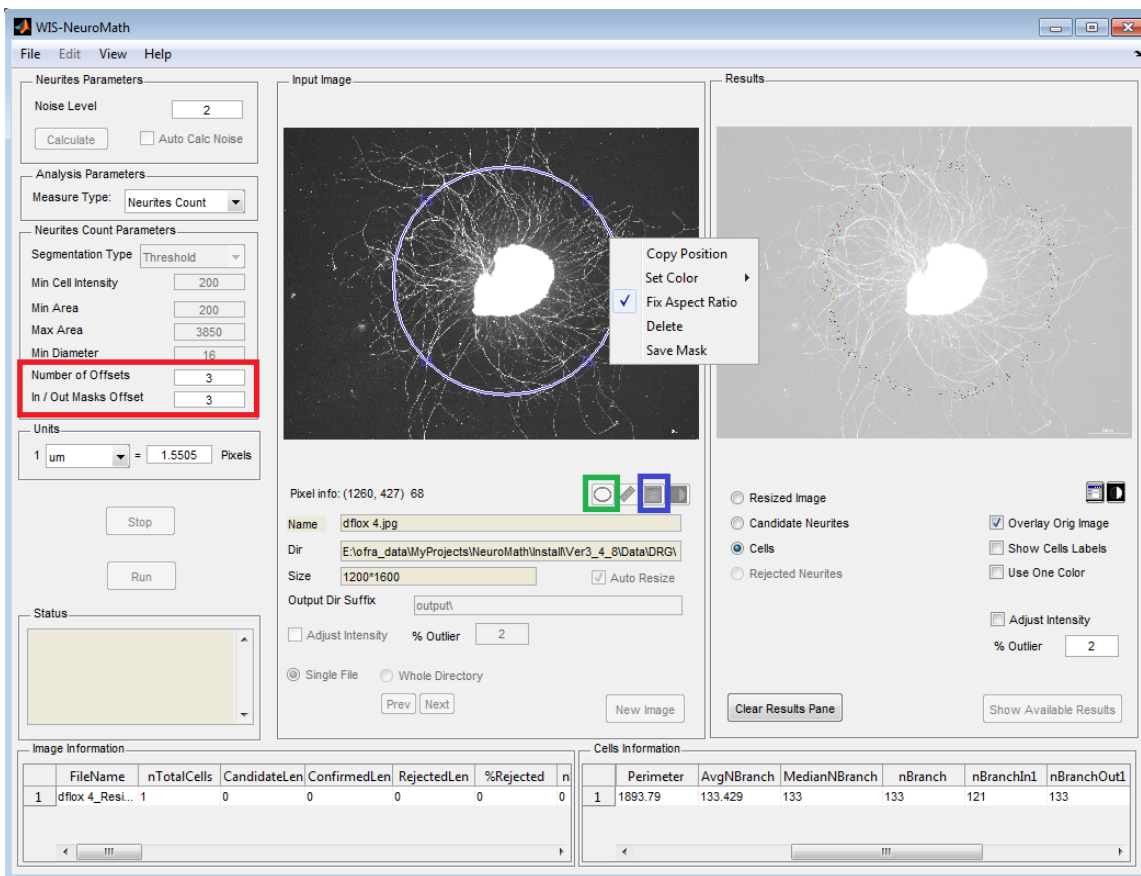


Figure 9: Neurite Count Setting

Once the mask is set you can run the analysis. You can set all the masks in advance and then run the quantification in a batch mode. The following calculated measures are shown in *Cell Information* table and written into the *Detailed Results File* includes. The Image Information table is not relevant for this module.



Parameter Name	Description
AvgNBranch	Average number of crossing neurites over all masks
MedianNBranch	Median number of crossing neurites over all masks
nBranch	Number of neurite crossing the defined mask
nBranchIn1	Number of neurite crossing the 1 offset inside the defined mask
nBranchOut1	Number of neurite crossing the 1 offset outside the defined mask
nBranchIn2	Number of neurite crossing the 2 offsets inside the defined mask
nBranchOut2	Number of neurite crossing the 2 offsets outside the defined mask
....	

Table 4: Neurite Count Measures

## **Total Outgrowth**

In order to get total outgrowth parameter use cell morphology mode. However, as the explant intensity varies a lot, you should define the explants “borders” manually using a mask.

Choose *Mask* instead of *Threshold* for the *Segmentation Type* in the Cell Parameters panel.

Use the  button to define a mask. You can do this either in the main window or in a separate window (use the  button). To save the mask click the mouse' right button. A context menu will be shown which let you delete or save the mask. The masks are saved into *Masks Sub Directory*, the name of the mask is set the file name with extension defined by *Mask File Suffix*. These values can be set using the Settings window (see Figure 10), which let you also set other default properties of the Mask. If you are using both total outgrowth and neurites count on the same image, use different *Mask File Suffix* for each of them.

Once the mask is set you can run the analysis. You can set all the masks in advance and then run the quantification in a batch mode. Overall image measurements are shown in the *Image Information* table on the bottom left side of the window, as detailed in the Quick Start section.

## **10. Preferences and Settings**

The *Settings* window allows you to control some additional application settings. Choose *Settings* from the *Edit* menu to set default Image Location, Results File names and format and color to grayscale conversion scheme (see Figure 10).

You can save all parameters and settings into preferences file, which can be loaded later on. Typically it is useful to have different preferences file for each experimental setup.

Use *Load Preferences* and *Save Preferences* from the File menu for this.

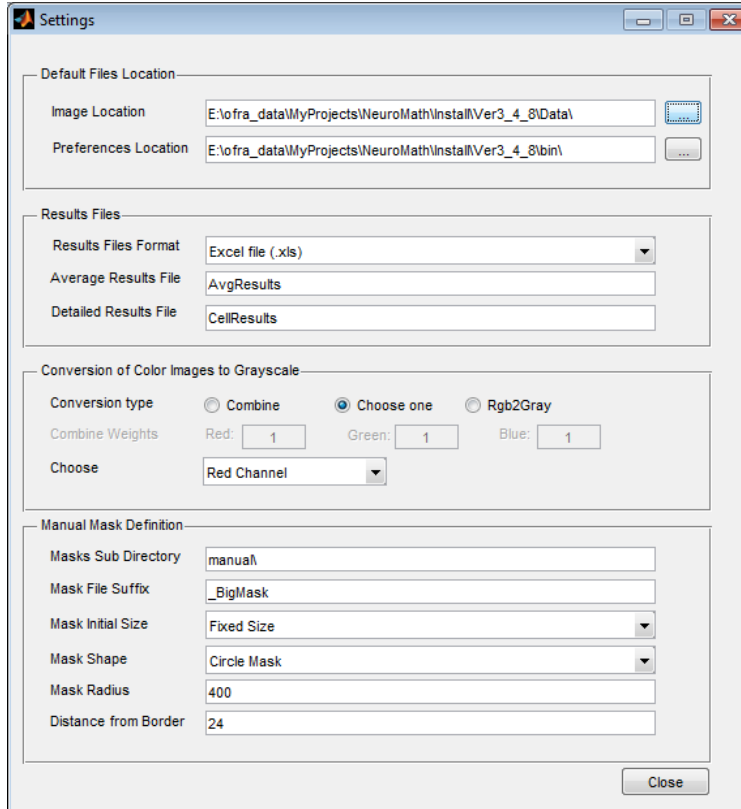



Figure 10: Settings Window

You can get back to the default parameter setting by choosing *Restore Default Parameters* from the File menu.

Choose *Restore Run Parameters* from the *File* menu to view and set the *WIS NeuroMath* parameters to the parameters used in the last run (in a given directory).

## 11. Working with high resolution images

High resolution (up to 16bit per pixel) images are supported. You can control the displayed range either using the *Adjust Intensity* checkbox and *%Outlier* value or by clicking *Adjust Contrast* button . A new window will open that will allow you to change the displayed data range. The easiest way is to use the mouse to drag the red lines to the desired value. The image is updated on the screen as you do it.

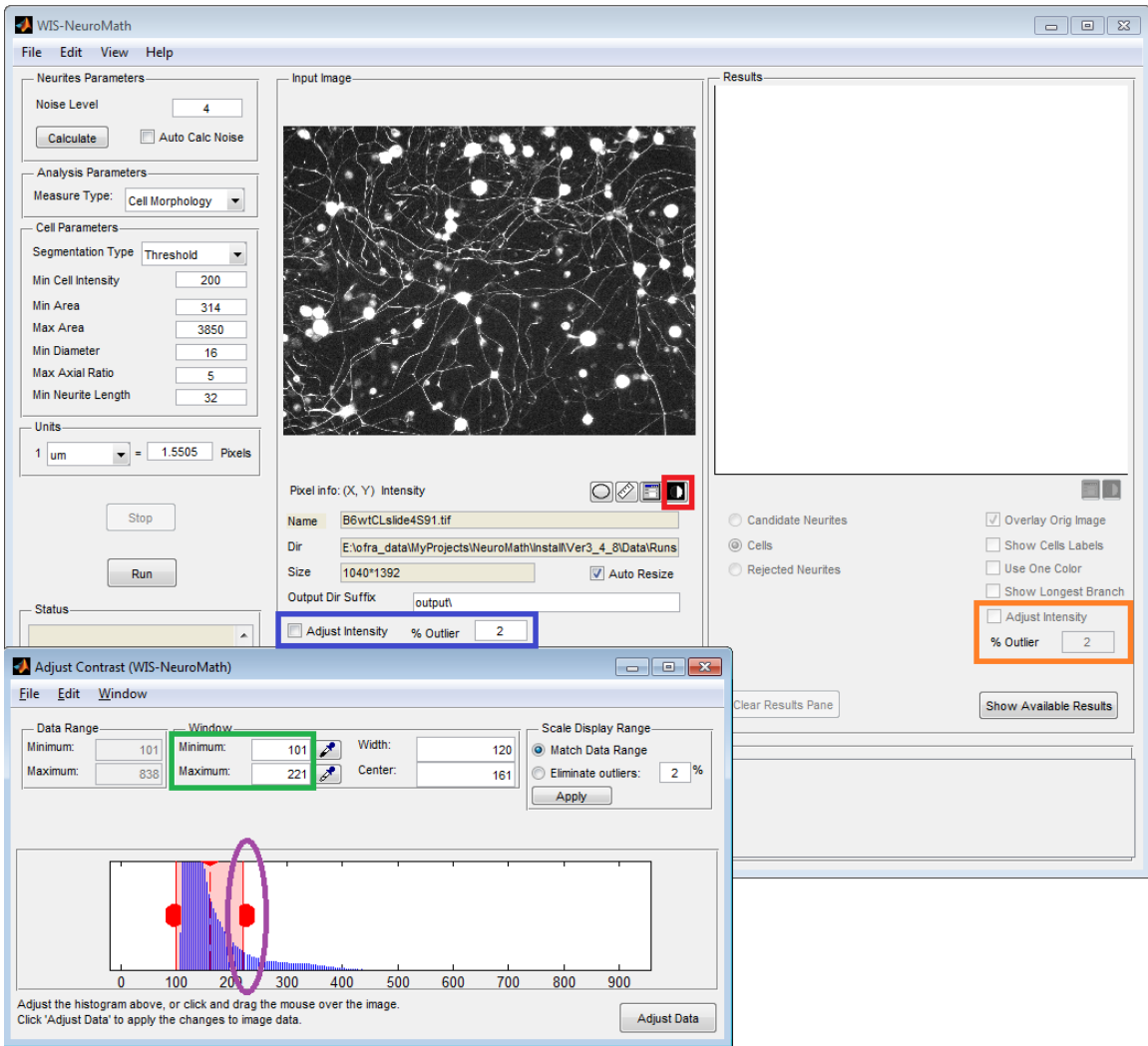


Figure 11: Adjust Contrast

Note that the current default parameters are set for low resolution images.

Set *Min Cell Intensity* value to proper value (e.g. 2500). You can use the *Pixel Info* tool for this (see chapter 12).

You might also want to change *Noise Level* to higher value (e.g. 8-10), but this depends on the image.



## 12. Measurement Tools

Several measurement tools and utilities are provided:

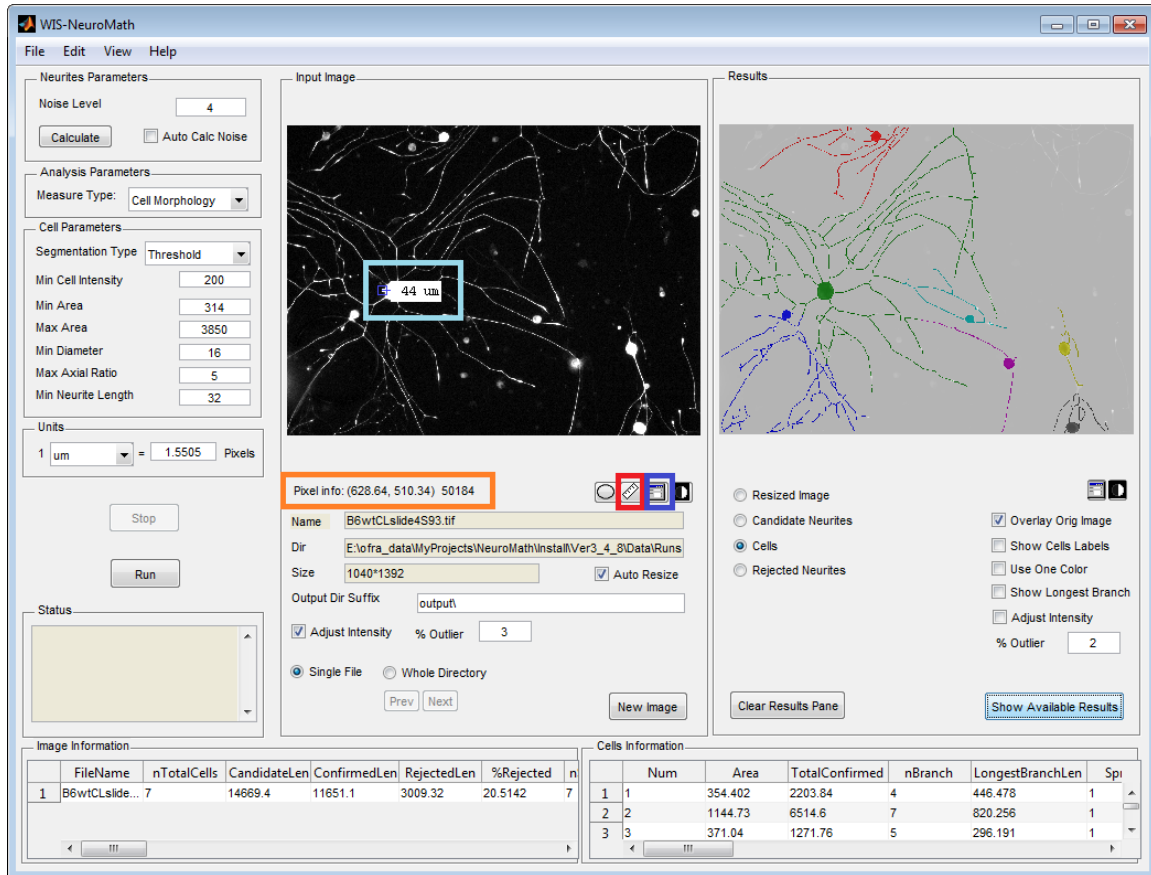



Figure 12: Measurement Tools

### Intensity Measure

When you move the mouse in the original image area, the pixel value at the current mouse location is shown in the *Pixel Info* field, just underneath the image. This is very useful for setting the *Min Cell Intensity* value. Intensity measure is more accurate in the separate window.

### Distance Tool

To measure cell diameter click on the *Distance Tool* icon . This will create a *Distance Tool* on the original image.

The *Distance* tool is a draggable, resizable line, superimposed on the image that measures the distance between the two endpoints of the line. Using the mouse, you can move and resize the *Distance tool* to measure the distance between any two points in an image. The


*Distance tool* displays the distance in a text label superimposed over the line. The tool specifies the distance in the units defined in the Units panel. Figure 12 shows a *Distance tool* on the original image. You can move the *Distance tool* over an image by dragging it with the mouse. You can also resize the *Distance tool* by selecting one of the endpoints with the mouse and dragging the endpoint.

The *Distance tool* has a context menu associated with it that allows you to

- Toggle the distance label on/off
- Specify horizontal and vertical drag constraints
- Delete the Distance tool object

Right-click to access the *Distance tool* context menu.

### Zoom-In

To view the image in a separate window, which you can resize easily, click the *Separate Figure* icon . *Distance tool*, *Adjust Contrast* and *Pixel Info* are provided for the separate figure as well.

### Color images

True color images are automatically converted to grayscale, as all the processing is done on grayscale images. You can choose the conversion scheme using the *Setting* window.

## 13. Miscellaneous

Images bigger than about 800\*800 pixels are automatically resized. If resize is done the resized image can be shown in the Results panel (above candidate neurites). The user should make sure that the neurites are well recognized by eye in the resized image. In general it is not recommended to use images that require resize factors over 3-4. On the other hand NeuroMath handles is more suited for detection of thin neurites (3-10 pixels wide). The user can adjust the neurite width by changing the Neurite Fiber parameters in the Advanced parameters window. However when working with large magnification where the neurites are much wider resizing the image can improve the performance.

Temporary result files are created in the *tmp* sub folder of output directory. This includes all the visual results and can take considerable disk space. If you no longer want the visual results, you can safely remove all files in the *tmp* sub folder, but make sure not to remove *AvgResults.txt* and *CellResults.txt* which contains the summary of results if you need them.

The results files are either Excel files (.xls) or comma separated files (.txt). To open comma separated files in Excel, Click *File* → *Open* from the Excel's main menu. In the dialog box go to the relevant folder, choose *All Files (\*.\*)* in the *Files of type* field to see the text files. A Text Import Wizard will open, choose the delimited radio button. And click the *Next* button. Choose *Comma* in the *Delimiters* panel and click *Finish*.

## 14. References

M. Galun, R.Basri and A. Brandt, Multiscale edge detection and fiber enhancement using differences of oriented means, ICCV, 2007.