Techniques

WIS-NeuroMath Enables Versatile High Throughput Analyses of Neuronal Processes

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ABSTRACT: **Automated analyses of neuronal morphology are important for quantifying connectivity and circuitry *in vivo*, as well as in high content imaging of primary neuron cultures. The currently available tools for quantification of neuronal morphology either are highly expensive commercial packages or cannot provide automated image quantifications at single cell resolution. Here, we describe a new software package called WIS-NeuroMath, which fills this gap and provides solutions for automated measurement of neuronal processes in both *in vivo* and *in vitro* preparations. Diverse image types can be analyzed without any preprocessing, enabling automated and accurate detection of neurites followed by their quantification in a number of application modules. A cell morphology module detects cell bodies and attached neurites, providing information on neurite length, number of branches, cell body area, and other parameters for each cell. A neurite length module provides a solution for images lacking cell bodies, such as tissue sections. Finally, a ganglion explant module quantifies outgrowth by identifying neurites at different distances from the ganglion. Quantification of a diverse series of preparations with WIS-NeuroMath provided data that were well matched with parallel analyses of the same preparations in established software packages such as MetaXpress or NeuronJ. The capabilities of WIS-NeuroMath are demonstrated in a range of applications, including in dissociated and explant cultures and histological analyses on thin and whole-mount sections. WIS-NeuroMath is freely available to academic users, providing a versatile and cost-effective range of solutions for quantifying neurite growth, branching, regeneration, or degeneration under different experimental paradigms.** Develop Neurobiol 00: 000–000, 2012

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INTRODUCTION

Automated quantification of different parameters of neuronal morphology is an increasingly important need in many neuroscience research projects. Manual analyses of neurite outgrowth are extremely time con-

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suming, even with the help of semiautomatic tools such as NeuronJ, a plugin for ImageJ (Meijering et al., 2004). A number of commercial tools allow automated measurements of neurite lengths and morphologies *in vitro* in cultures, including MetaXpress from Molecular Devices, INCell Analyzer from GE Healthcare, and HCA-Vision from the Australian CSIRO (Wang et al., 2010). These software packages provide cell-by-cell measurements such as total outgrowth, cell body area, and branching, with minimal human operator intervention, but their disadvantages include different degrees of ease of use and pricing that limits their availability.

Freewares available to the academic neuroscience community include NeuriteTracer (Pool et al., 2008), a freely available fully automatic plugin for ImageJ, which measures the total neurite lengths in an image and counts the number of neuronal nuclei but does not trace the neurites to attach them to individual cells. Thus, it provides population-averaged data, and cannot reach individual cell resolution. Similar limitations apply for the method described by Wu et al. (2010). NeuronCyto is another freeware that uses a segmentation-based approach validated on N1E115 mouse neuroblastoma cells (Yu et al., 2009). However, it is limited to images in which neurites of different cells do not intersect, which restricts its utility for analyses of cultured primary neurons. Other approaches described in the literature are usually focused on individual applications, such as quantification of neurite arbors from *in vivo* sections (Broser et al., 2008; Helmstaedter et al., 2011). An alternative approach is to extract neurite-representing pixels to determine ratios of such pixels from different sides of an image, thus defining relative degrees or directions of outgrowth (Rosoff et al., 2004; Mortimer et al., 2009). A recent extension of the latter approach demonstrated ellipse-fitting measurements of neurite outgrowth from explants to investigate the effects of trophic factor gradients (Haines and Goodhill, 2010). There is a wide range of tools for other types of neuronal cell analyses, such as neurite growth rate, synapse morphology, 3D cell morphology reconstruction, etc. For instance, NeuronGrowth (Fanti et al., 2011) is a freely available semiautomatic plugin for ImageJ, which allows semiautomatic tracking and measurement of the selected neurites along a time-lapse sequence. This plugin can be helpful in growth rate kinetic analysis of individual neurites but not for high throughput screens of cell morphology. Synapse Detector (SynD) is a freely available tool for synapse morphology, and its localization detection feature provides per image information (Schmitz et al., 2011) but not single cell resolution.

Overall, the currently available tools for quantification of neuronal morphology are either highly expensive commercial packages, some of them are restricted to proprietary imaging platforms and instrument-specific image formats, or freewares that cannot provide automated image quantifications at single cell resolution. To fill this gap, we have developed and validated a new software tool for high throughput analyses of neuroscience images, called WIS-NeuroMath. WIS-NeuroMath is based on an algorithm capable of efficient multiscale detection of edges and fibers in two-dimensional images (Galun et al., 2007), allowing direct and accurate detection of neurites in various conditions. Here, we demonstrate the utility of WIS-NeuroMath for a range of applications, including quantification of neurite outgrowth in primary neuron cultures, counting and measuring neurite extension from an explant, and detection and analyses of neurite morphology from sections of *in vivo* preparations.

METHODS

Algorithm and Overview of the Procedure

WIS-NeuroMath is based on accurate detection of candidate neurites using the algorithm described by Galun et al. (2007), which does not require any image preprocessing. Using a scale adaptive threshold, this algorithm first reveals edges by identifying stretches of statistically significant contrast along lines of different lengths and orientations. Edges are localized accurately even when contrast is low and in very noisy images. Then, by utilizing a stochastic completion-like process from the whole set of detected edges, even very faint fibers are detected. Following this initial detection step, different types of processing can be carried out depending on the desired application. For example, neurite outgrowth quantification in culture first requires cell morphology analysis to detect cell bodies, after which the candidate neurites are traced to allow assignment to the connected cell bodies. Neurite lengths, branching, cell body area, and other parameters are then calculated and saved for each cell. 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 (for example, fine tuning of neurite detection according to image resolution and background level), to perform analyses on single files or whole directories, and to browse through the results. Comparisons of WIS-NeuroMath results to manual quantifications in NeuronJ (Meijering et al., 2004) were carried out independently by three different users. For further details on the software, please see http://www.weizmann.ac.il/vet/IC/software/wis-neuromath.

Cell Morphology of Cultured Neurons

The same image serves to identify both neurites and neuronal cell bodies, the latter detected using simple threshold-based segmentation. Neurite tracing is treated as a graph-

search problem. The candidate neurites are defined as all the detected thinned fibers, excluding cell bodies. They are represented as an undirected graph, in which each neurite pixel is a node with edges to its eight-connected neighbors that are detected as neurites. Each edge is associated with the Euclidean distance between the two pixels.

Seed nodes of a cell body are defined as the pixels in which the candidate neurites cross the cell body perimeter. The number of seed nodes is thus the number of the main branches that exit the cell bodies, and they serve as starting points for neurite tracing. Neurite tracing is done by using the Dijkstra algorithm (Cormen et al., 2001) to calculate the minimal distance from any seed node to any other node in the graph. Each node in the graph is associated with the closest seed node, thus each pixel is associated with its nearest seed point and with the cell that it belongs. This ensures that each neurite pixel is associated with the appropriate cell or not associated at all if a viable connection does not exist in the image. If neurites of two cells cross over each other, the ambiguity is resolved by dividing the crossing neurites between the two cells at the point of equal distance between the cells. Users should be aware of this limitation when conducting analyses of highly dense cultures. Since, in this case, the total outgrowth parameter of individual cell is not reliable, average total outgrowth or average of sprouted cell total outgrowth parameters from per image measurements is recommended.

Once neurite detection and labeling (assignment to connected cell bodies) are complete, unlabeled neurites are discarded. Depending on the type of culture, the unlabeled neurites may represent debris from dying or injured neurons or processes originating in neuronal cell bodies not included in the field of view. The Cell Morphology mode by default removes all neurites not connected to cell bodies from the cell total outgrowth parameter. Total outgrowth including unconnected neurites is available as the Candidate Neurite Length parameter. The following parameters are then calculated per cell: cell body area, number of main branches, total outgrowth (the length of all neurites trees attached to the cell), and longest branch (the point most distant from any of the cell's seed nodes). In addition, the length of the neurite tree originating in each seed node, and the length of the longest branch for each seed node is calculated, and the software then calculates average, median, and maximum values for each. Other per cell parameters provided by the software include average cell body intensity, average neurites intensity per cell, and neurite outgrowth status (e.g., the fraction of neurite bearing cells in a population) based on a minimum neurite length threshold set by the user. Per-image measures include data such as total number of cells, percent of outgrowth, and average total outgrowth per cell. Individual cell data are exported to Excel files, as well as image averages.

Neurite Length Analysis on Sections

This module of the software provides a solution for images in which cell bodies are not present, for example, longitudinal nerve sections that contain only neurites and nonneuronal 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 detection in certain experiments. The number of unconnected neurite elements and their average length is calculated together with the median and 70th, 80th, and 90th percentile lengths, enabling quantitative evaluation of *in vivo* neuron degradation and regeneration. For other experimental paradigms resulting in images of neurites without cell bodies, the software measures additional parameters such as the number of branch points and the branching complexity (number of branch points divided by the neurite length). These parameters can be useful in arborization stage of axon development during embryogenesis to distinguish between normal and deficiency conditions in histological analysis of whole-mount sections.

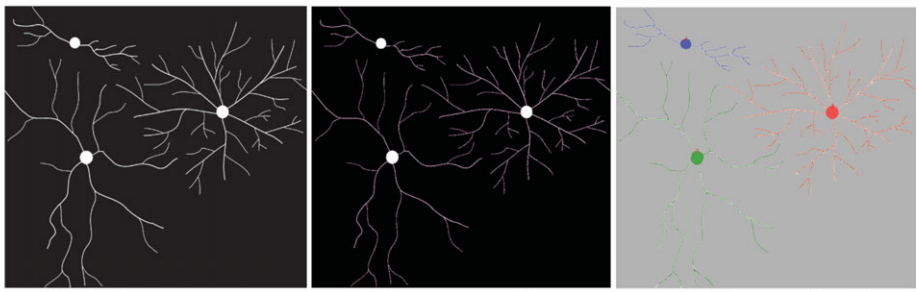
Ganglion Explant Analysis

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. Here, we tried to resolve this problem using two approaches. In the first approach, calculation of process number is carried out at different distances from the ganglion, allowing distinction between processes that separate from each other as they grow out. Although this mode does not trace individual processes, the number of identified axons in a distance series provides information about the degree of fasciculation and explant morphology. This tool bears some resemblance to classical Sholl analyses (Sholl, 1953) of dendritic arbors. Technically, in this mode, the user manually defines an ellipse mask around the ganglion, and the software then detects neurites and counts the number of neurites crossing that mask. The software can calculate 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. The second approach for quantification of explants utilizes a total outgrowth parameter, based on explant images that include all processes in the field of view [Fig. 3(A)]. The morphology module of the software can then be used after manual application of a mask on the ganglion.

Neuronal Preparations and Imaging

A variety of different types of neuronal preparations were used to test and optimize the software, including adult sensory neuron cultures in regular dish format (Hanz et al., 2003) or on filter inserts to discriminate neurites from cell bodies (Zheng et al., 2001), longitudinal sections of regenerating peripheral nerve (Perlson et al., 2005), embryonic mouse (E14.5) paw whole mounts (Wickramasinghe et al., 2008), and explant cultures of embryonic (E13.5 or E18) mouse dorsal root ganglia (DRG) (Schoenmann et al., 2010). Imaging was carried out on a Nikon eclipse Ti-S fluorescence microscope at 10× or

A



B

MA=Manual Analysis NM=NeuroMath

	Total Length			Avg Process Length			Cell Body Area			n Processes		
Cell	MA	NM	%diff	MA	NM	%diff	MA	NM	%diff	MA	NM	%diff
1	2263	2309	1.9	1131	1155	2.0	4988	4833	-3.1	2	2	0
2	7709	7909	2.6	1927	1977	2.6	7325	7497	2.3	4	4	0
3	11646	11831	1.6	1941	1971	1.5	6663	6912	3.7	6	6	0

Figure 1 Validation of WIS-NeuroMath on model neuron images. A: Manually drawn images of neuronal cells (left) were analyzed with NeuronJ (center) compared to automated neurite detection with WIS-NeuroMath (right). B: Quantification of total neurite outgrowth, cell body area, average process length, and number of processes per cell shows good agreement of both methods. Data quantification by WIS-NeuroMath proved to be robust under different parameter settings. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

4× magnifications with Nikon objectives (NA 0.3 and NA 0.2, respectively), on an ImageXpress Micro (Molecular Devices) at 10× magnification with Nikon objective (NA 0.3), or on an Olympus IX81 FlowView 1000 confocal microscope at 4× or 10× magnifications with Olympus objectives (NA 0.16 and NA 0.4, respectively). Confocal stacks from embryonic limbs were acquired at 10–15 μm intervals and digitally collapsed. The obtained images were 8 bit for Nikon eclipse Ti-S and Olympus

Figure 2 Quantification of neuronal process morphology in culture. A–C: Batch analysis of a conditioning lesion experiment using either WIS-NeuroMath or MetaXpress (Molecular Devices). A. Fluorescent images of DRG neuron cultures from control mice (upper panels) versus 3 days after sciatic nerve lesion (lower panels). Representative detection and tracing in both software packages did not show any significant differences in cell number, total outgrowth, or cell body area. The insertions represent overlay of software tracing on original image. B: Batch analysis of control and injury groups revealed 2.5-fold versus 2.9-fold increases in total outgrowth after injury as quantified in WIS-NeuroMath (NM) versus MetaXpress (MDC), respectively. C: Quantifications of cell body areas were very similar in both software packages. ***$P < 0.001$ ($n = 500$; Student's t test,), "n" indicates number of images. D–F: Comparison between WIS-NeuroMath and MetaXpress batch analyses of the same 500 neuron-culture images for cell number (D), total outgrowth (E), and cell body area (F). Since neuronal soma are not perfect circles, both programs will err somewhat in this measurement, and the weaker correlations for this measurement are most likely due to differing degrees of error arising from varying geometries of the cell bodies. Spearman's correlation coefficients are indicated. Scale bar is 100 μm. G,H: Measurements of neurite outgrowth using WIS-NeuroMath on images of sensory axons from compartmentalized filter cultures. G: Axons of DRG neurons from control (upper panels) versus 3 days after sciatic nerve lesion (lower panels) after 72 h in culture on filters with 3-μm diameter pores. Images are from the lower side of the filter, showing processes only without cell bodies, original images on the left, and WIS-NeuroMath neurite tracings on the right. H: Batch analysis of axon outgrowth revealed a 1.7-fold increase in neurite length after injury, confirming the conditioning lesion effect ($n = 300$), n indicates number of images. Scale bar is 100 μm. [Color figure can be viewed in the online issue, which is available at wiley onlinelibrary.com.]

confocal microscopes and 12 bit for ImageXpress Micro high throughput acquisition.

RESULTS

Validation of WIS-NeuroMath on Model Neuron Images

WIS-NeuroMath is based on the algorithm of Galun et al. (2007), which was previously validated on a series of test images of different types. To confirm this validation on model neuron type images, we generated artificial test images of neurons with increasing

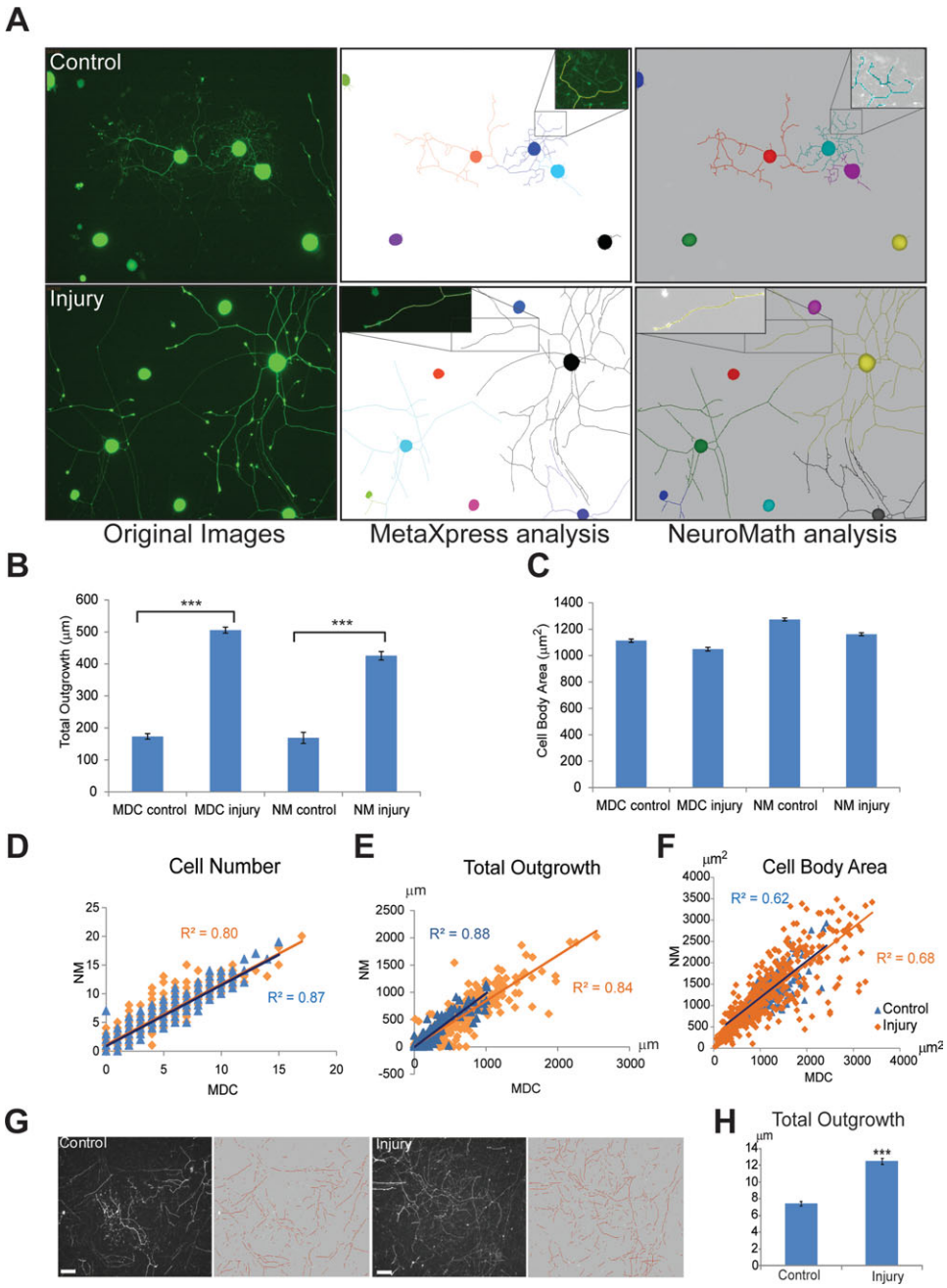


Figure 2

neuritic arbor complexity and quantified them using NeuronJ (Meijering et al., 2004) and then analyzed the images using WIS-NeuroMath under a range of parameter settings (Fig. 1). The results confirm that the software provides accurate morphology measurements of artificial neuron images and, moreover, that these measurements are robust over a broad range of parameter settings. We, therefore, progressed to challenge WIS-NeuroMath with a range of images from different types of neuronal preparations.

Analysis of Neurite Outgrowth in Cultured Neurons

The "conditioning lesion" paradigm (Smith and Skene, 1997) is widely used to examine regeneration ability of peripheral sensory neurons. Sensory ganglia are composed of diverse neuronal subpopulations with differing sizes and morphologies, requiring high numbers of cell measurements to obtain precise results. We compared the cell morphology module of WIS-NeuroMath to MetaXpress, a commercial software package from Molecular Devices, for measurement of neurite outgrowth and cell body area in cultured mouse DRG neurons with and without conditioning lesion. The DRG neurons were acquired on an ImageXpress Micro imaging system at 10× magnification in order to obtain all neurite trees in one field of view, allowing more reliable analysis of total outgrowth. Figure 2 demonstrates performance of the two automated image analysis programs in this task. Both programs detected and quantified significant increases in neurite outgrowth in conditionally injured neurons compared to control [Fig. 2(A,B)] and slight decreases in cell body area [Fig. 2(C)]. WIS-NeuroMath and MetaXpress results correlated well for number of cells per image [$R^2 \geqslant 0.80$, Fig. 2(D)] and for average total outgrowth per image [$R^2 \geqslant 0.84$, Fig. 2(E)]. Cell body area measurements showed lower correlation [$R^2 =$ 0.62–0.68, Fig. 2(F)], most likely due to differences between the software packages in handling the nonperfect circular shape of the cell bodies. Thus, WIS-NeuroMath showed comparable performance in neurite morphology quantification to a leading commercial software, while having clear advantages in terms of use and flexibility.

In another test of WIS-NeuroMath capabilities for quantification of neurites in culture, we examined the neurites-only surface of a compartmentalized filter culture of DRG neurons, with and without prior conditioning lesion. The images were obtained using ImageXpress Micro at 10× magnification. Figure 2(G) shows 72-h cultures immunostained with anti-β-tubulin (left), and neurites detected by WIS-NeuroMath (right). The program shows fine capability in capturing essential features of the images, and the automated quantification reveals the expected differences in neurite outgrowth caused by the conditioning lesion [Fig. 2(H)].

Axon Outgrowth From Explants

Dissociated cultures are convenient for analyses of neuronal growth, but unfortunately, they do not preserve the normal relationships between neurons, glia, and other cell types in a tissue. Thus, ganglia or tissue explants are preferred for some applications, and axon guidance or growth studies with explants of peripheral ganglia can require quantification of profuse neurite extension, such as in the case of the E18 DRG explant 48 h in culture shown in Figure 3(A,B). The images were acquired using a Nikon eclipse Ti-S fluorescence microscope at 4× magnification in order to capture the entire length of neuronal processes. We tested the utility of the cell morphology module of WIS-NeuroMath for this type of preparation, using a manually defined circle or ellipse-shaped mask to encircle the ganglion, providing start points for neurite length measurements [Figure 3(A,B)]. One can define multiple masks in advance and then run analyses in batch mode. We also developed a ganglion analysis mode to count the number of neurites crossing a manually defined mask [Fig. 3(C)] and compared the results of manual counting versus WIS-NeuroMath counts on a series of images using the same masks. Manual counts were performed for comparison over nine images using ImageJ. The automated counting results correlate very well with manual counts [Fig. 3(D,E); $R^2 = 0.96$]. In this case, the images were taken from an E13 DRG explant using 10× magnification.

Analyses of Neurites on Histological Sections

Sections from *in vivo* preparations are typically more difficult for automated image processing than from cultured neurons and are often analyzed manually, requiring enormous investments of researcher time. We tested the versatility of WIS-NeuroMath in analyses of two different types of tissue-derived images, quantification of axonal degeneration or outgrowth after peripheral nerve injury (Fig. 4) and quantification of neurite morphology and branching in whole-mount immunostaining of embryonic paws (Fig. 5).

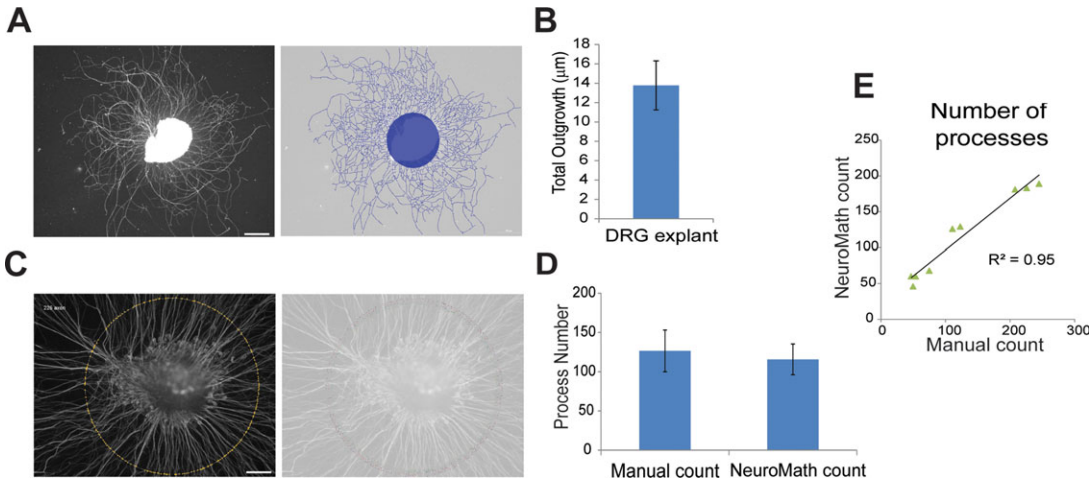


Figure 3 Quantification of axon outgrowth from explants. A: Fluorescent images of DRG explants from E18 mouse embryos cultured for 2 days, then fixated, and immunostained for neurofilament heavy chain (NFH). The morphology mode of WIS-NeuroMath was used to measure total outgrowth and number of main branches. A user-set circular mask around the ganglion provides start points for neurite length measurements. Scale bar: 200 μm. B: Batch analysis of nine images from the experiment of A to quantify total outgrowth. C: Fluorescent images of DRG explants from E13.5 embryos after 2 days in culture followed by NFH immunostaining. The DRG Analysis mode of WIS-NeuroMath was used to count the number of neurites crossing a manually defined mask (right), as compared to manual counting (left). Scale bar: 100 μm. D,E: Comparison of manual versus automated counts of process numbers per ganglion ($n = 9$), n indicates number of images. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

During the course of a study on axonal responses to peripheral nerve lesion, we carried out crush injuries of the sciatic nerve in transgenic mice that express yellow fluorescent protein (YFP) in sensory neurons (Feng et al., 2000) and used the neurite length analysis module of WIS-NeuroMath to detect and quantify YFP-labeled neurites on longitudinal sections from the injured nerves [Fig. 4(A,B)]. The images were taken using a Nikon eclipse Ti-S fluorescence microscope at 10× magnification along the nerve, moving from the injury site in both directions. Analysis of images from different time points after injury showed extensive axonal fragmentation around the lesion site up to 8 days after injury, with recovery and appearance of longer and increased numbers of axonal processes in section planes at later time points [Fig. 4C,D)]. WIS-NeuroMath thus provides a completely automated image analysis solution for image types that would otherwise require extremely laborious manual or semiautomatic processing (e.g., Tuszynski et al., 2002; Kurimoto et al., 2010).

An additional class of images that pose a challenge for automated analyses is whole-mount immunohistology. We tested the utility of WIS-NeuroMath in analyses of neuron branching in embryonic limbs by analyzing whole mount anti-peripherin immunostaining on paws of E14.5 mice. After staining of the limbs, dorsal surfaces of paws were imaged from whole-mount preparations, using an Olympus confocal microscope at 4× magnification. Total outgrowth of sensory processes within the fields of view was quantified by WIS-NeuroMath. Representative images with automated neurite detection by WIS-NeuroMath compared to manual tracing with NeuronJ are shown in Figure 5(A). Processing of such an image by WIS-NeuroMath on a standard Windows PC took less than 20 s, allowing processing of over 200 images per hour. Manual tracing of such an image in NeuronJ by a human operator would require 5–10 min per image, depending on tracing skill and focus ability of the researcher. Automated and manual analyses were in good agreement [Fig. 5(A,B)], with a correlation coefficient R^2 of 0.75 for total axon lengths measured in both methods [Fig. 5(C)]. Thus, WIS-NeuroMath allows vastly increased throughput of histological images without sacrifice of analysis quality. Since the program detects edges by comparisons of adjacent pixels regardless of detection method, there is no bias or requirement for any specific type of staining or visualization.

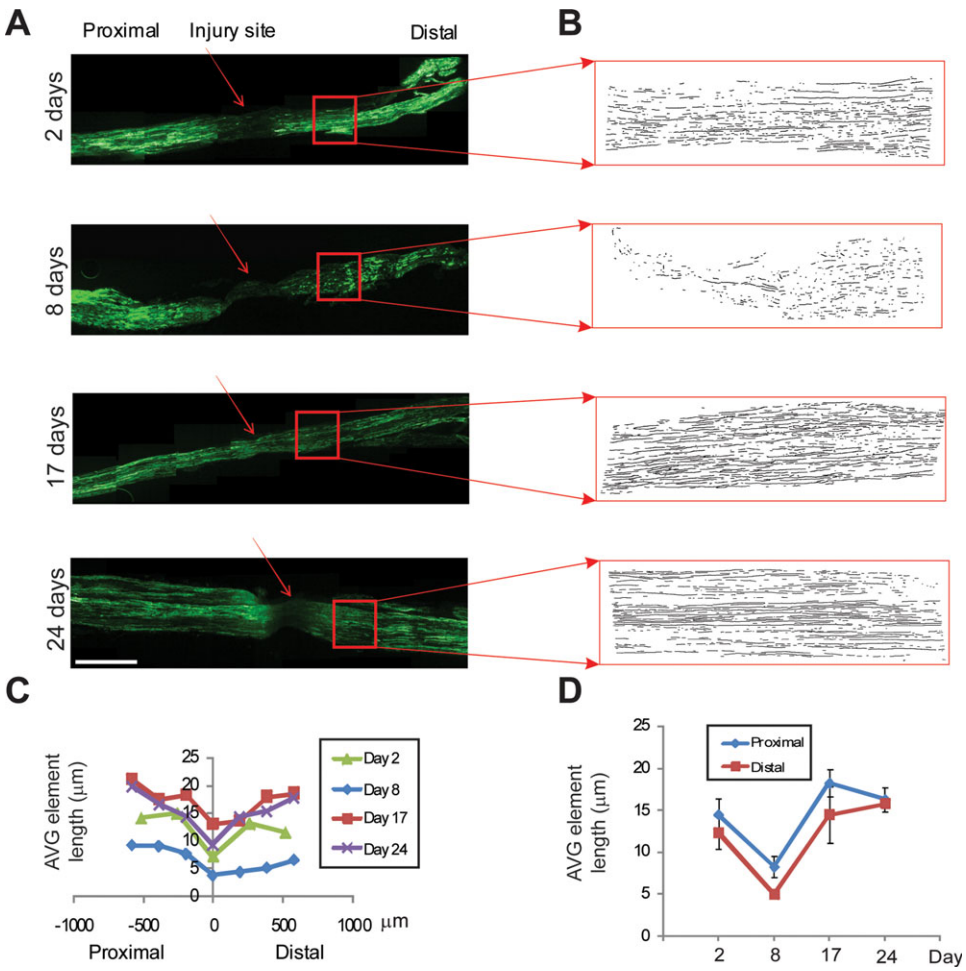


Figure 4 Analysis of peripheral nerve regeneration: (A) fluorescent images and (B) WIS-NeuroMath neurite tracings (right) from longitudinal sections of sciatic nerves from YFP16 transgenic mice 2, 8, 17, and 24 days after crush injury. Three sciatic nerves were analyzed for each time point. Sections (100 μm) distal to the injury site shown. C: Quantification of average axon fragment lengths at the indicated positions up to 600 μm proximal or distal from the injury site at all four time points. The analysis reveals an overall reduction in axonal fragment lengths up to Day 8, indicative of postinjury degeneration. Subsequent time points show increases in axon fragment lengths, with regrowth completed by Day 24. These kinetics are similar to those reported in the literature using conventional measures of regeneration and repair. D. Time course display of the experiment, quantifying up to 500 μm proximal or distal from the injury site. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

Quantitative analyses of neuronal morphology is important for characterizing connectivity and circuitry *in vivo*, as well as allowing high content imaging approaches using primary neuron models *in vitro*. We have described how WIS-NeuroMath provides solutions for automated quantification of critical parameters in both *in vivo* and *in vitro* preparations. WIS-NeuroMath was tested on different computers running Microsoft Windows with at least 2GB of RAM memory. For example, an analysis capacity of 200 images per hour was obtained using an Intel Xeon 2.5GHz CPU on 12 bit images of 1392 $\times$ 1040 pixels, similar to those shown in Figure 2. Images bigger than 800 $\times$ 800 are automatically resized, and the measurements are scaled accordingly. WIS-NeuroMath is a flexible tool with user-friendly intuitive interfaces for parameter setting and usage and can analyze a broad range of image types and formats in single files or in automated high throughput mode.

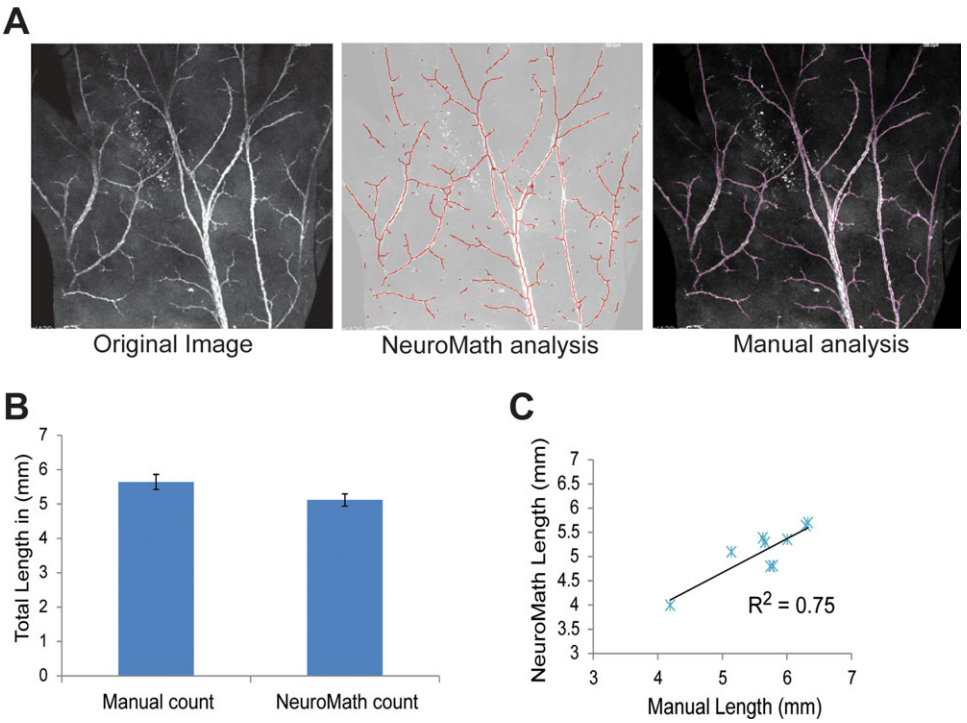


Figure 5 Quantification of neurite lengths during development in vivo. A: Visualization of axonal projections in whole mounts of front paws from E14.5 mouse embryos (left), with automated neurite detection of the image by WIS-NeuroMath (center) compared to manual tracing using NeuronJ (right). B,C: Quantification of total axonal length per paw over nine preparations shows good agreement of both methods. Spearman's correlation coefficients are indicated. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The advantage of the WIS-NeuroMath software suite over other solutions described above is its combination of versatility and broad range of applications together with its cost-effectiveness. In addition to the applications described above, it has been used and validated in a number of recent studies (Ma et al., 2011; Ben-Yaakov et al., 2012; Perry et al., 2012; Rishal et al., 2012). The software package is freely available to academic users under a standard MTA and can be downloaded from a dedicated web page on the Weizmann Institute website (http://www.weizmann.ac.il/vet/IC/software/wis-neuromath) and installed on any personal computer with a Windows operating system. An intuitively approachable graphical user interface provides easy entry to the program and rapid implementation. Support is available via e-mail to wisneuromath@gmail.com, and requests and suggestions for further expansion or modification of the software are welcomed. We expect WIS-NeuroMath will prove to be useful not only in neuroscience but also in other fields requiring fiber measurements, such as analyses of circulatory systems.

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