SUPPLEMENTARY MATERIALS AND METHODS

Automated myelin quantification

The following is a detailed description of how the images were processed and the myelin was identified. CEM was developed to automate these operations. Its outputs include the resulting images and calculated values for each parameter as well as myelination percentages.

Generation of binary image stacks: First, the confocal stacks were split into single channel stacks (MBP-, TUJ1-, GFP- and DAPI-stacks). The brightness of each stack was adjusted to maximize the signal-to-noise ratio. A reference slice, one with a large amount of positive signal and low background noise, was selected for each stack independently. The binary conversion was done using ImageJ's built-in "Make Binary" function using IsoData method and dark background. Instead of calculating thresholds for each slice, the values from reference slice were used for the entire stack. The resulting image has two pixel values, 0 for black and 255 for white.

Cell body removal: We used "AND" operator of the built-in "Image Calculator" (ImageJ) to identify nuclei of oligodendrocytes (Binary-DAPI-stack "AND" Binary-MBP-stack) and of neurons (Binary-DAPI-stack "AND" Binary-TUJ1-stack). The resulting images were cleaned of noise via the "Despeckle" function and the multiple nuclei were divided into single nuclei via the "Watershed" function. We called these stacks of images Oligodendrocyte-Nuclei and Neurons-Nuclei, respectively. The MATLAB CEM GUI was used to remove particles whose area was smaller than 50 pixels square (using the MATLAB function "exclude") to eliminate nuclei partially overlapping with cell bodies. The remaining nuclei were dilated for 5 pixels to grow the nuclei into cell bodies using the MATLAB "imdilate" function. The 5-pixel dilation was performed by using a disk of radius 5 across the image. The resulting images were called Oligodendrocyte-Cell-Bodies and Neurons(plural?)-Cell-Bodies. Next, using ImageJ, cell bodies images were "Inverted" (ImageJ function), i.e., all 0 pixel values were turned into 255 and vice versa. When this image was "Multiplied" (an operator in ImageJ's "Image Calculator") to the corresponding binary image, i.e., Oligodendrocyte-Cell-Bodies "Multiplied" to Binary-MBP-stack, the result was the cell bodies removed (CBR-MBP-stack) image.

Identification of subsets of axons: Similar to nuclei identification "ANDing" binary TUJ1-stack (CBR or not) with the binary GFP-stack resulted in GFP-positive neurons (Marker-positive-axons). Next, we "Inverted" the Marker-positive-axon image and "Multiplied" it back to a binary TUJ1 image to identify nonGFP neurons (Marker-negative-axons).

Identification of myelin: We defined myelin as the overlap between the binary oligodendrocyte image and binary axon or subset of axon images. The overlap was identified via the "AND" operator. The total myelin for the entire device was identified by "ANDing" binary CBR-MBP-stack with the binary CBR-TUJ1-stack and resulting in CBR-Overlap-All image. The myelin for GFP and nonGFP axons was identified by "ANDing" binary CBR-MBP-stack to Marker-positive-axons and Marker-negative-axons, respectively.

Calculation of results: The results were generated as pixel counts and percent values. The pixels were counted using the "Histogram" function of ImageJ on maximum intensity projections. For example, applying "Histogram" function on CBR-TUJ1-stacks gave the number of TUJ1-positive pixels after cell body removal, which was defined as all axons here. While applying the "Histogram" function on maximum intensity, projected CBR-Overlap-All gave the total pixel count of myelin. The percent of myelinated axons was calculated by dividing the latter by the former and multiplying by 100. Similar calculations were performed for other values.

Manual myelin quantification

The length of each myelin segment was measured using the "Segmented Line" tool on ImageJ (NIH) for the entire myelination compartment. The lengths in pixels were summed for each sample, giving the total myelin of that sample. Total myelin for each condition was averaged and the average value for +T3 was used for normalization, as described in the manuscript.

Overlap Probability Calculations

We first determined the probability of a pixel being MBP positive P(MBP) and the probability of a pixel being TUJ1 positive P(Axon) within the myelination compartment independently for each experiment. The joint probability of a pixel being positive for both due entirely to chance was calculated as P(MBP)*P(Axon). We compared this chance probability for the +T3 condition to the myelin ratio observed for +T3 experimentally P(MBP | Axon) and these were significantly different ($p=0.0025$, N=11, Wilcoxon Ranksum). Similarly, the chance overlap in the -T3 condition as compared to the overlap observed from the -T3 experimental data was significantly different ($p=0.0079$, N=5, Ranksum), suggesting the myelination effects observed were not due to chance. Additionally, this method allowed us to examine how much the myelin overlap calculation was sensitive to changes in the MBP amount. To explore this question, we first determined the percent difference in MBP positive pixels in the +T3 and -T3 conditions (mean difference = 15%). Following this, the total number of MBP positive pixels was reduced by this amount so that the population's MBP means were now equal for +T3 and -T3. From this, we compared the myelin overlap for the "equalized T3" and the original -T3. Again, we found that these groups were significantly different ($p=0.0192$, N=11 T3, N=5 non-T3, Wilcoxon Ranksum), suggesting that the changes in myelination observed could not be accounted for due to changes in the MBP alone.

Cell culture methods and media compositions

Mouse ESC Medium consisted of, KnockOut DMEM (Life Technologies), 15% KnockOut serum replacement (Life Technologies), GlutaMAX (Life Technologies), 1x non-essential amino acids (Gibco), and 55 µM 2-mercaptoethanol (Gibco).

NPCs were generated and differentiated as described below that was modified from a previous study (Marchetto et al., 2008). ESCs were grown in suspension in mouse ESC

Medium without LIF for the first day and in N2/B27 Medium supplemented with 500 ng/ml Noggin (PeproTech) for four more days. Next, embryoid bodies were dissociated, plated on and maintained on laminin- (Life Technologies) coated dishes in N2/B27 Medium supplemented with 20 ng/ml Epidermal growth factor (EGF;PeproTech), 20 ng/ml Fibroblast growth factor-basic (bFGF; Stemgent).

N2/B27 Medium: DMEM/F12-Glutamax Medium (Gibco), 1x N2 supplement (Gibco), 1x B27 supplement (Gibco).

Differentiation Medium: N2/B27 Medium supplemented with 10 ng/ml Insulin-like growth factor 1 (IGF-1; R&D Systems), 10 ng/ml Platelet-derived grow factor-α (PDGF-α; R&D Systems).

Maturation Medium: N2/B27 Medium supplemented with 10 ng/ml Ciliary neurotrophic factor (CNTF; Sigma-Aldrich), 5 ng/ml Neurotrophin-3 (NT3; Sigma-Aldrich) + 40 ng/ml Triiodothyronine (T3; Sigma-Aldrich).

Neuron Medium, which was described before (Gaspard et al., 2009), consisted of, 1:1 DDM Medium:Neurobasal/B27 Medium supplemented with 20 ng/ml Glial cell derived neurotrophic factor (GDNF; R&D Systems), 500 µg/ml cAMP (Sigma), and 0.2 µM ascorbic acid (Sigma).

Microfluidic devices

Microfluidic devices were fabricated as described previously (Taylor et al., 2005). Two layers of SU-8 photoresist, 3 µm high microgrooves and 100 µm high channels, were generated on a silicon wafer by using standard photolithography techniques. To fabricate microfluidic devices, 1:10 ratio of curing agent and poly-dimethly-siloxane (PDMS, Sylgard 184, Dow Corning Co.) was mixed and cured on that master mold. The enclosed channel on one side was cut out to achieve the open well design (Fig. 4A; supplementary material Fig. S3B). The microgrooves were 10 µm wide, 3 µm high and 150 µm long. Glass coverslips (Fisher Scientific) were coated with 0.5 mg/mL poly-L-lysine (Sigma); then the devices were assembled onto coverslips, followed by coating with laminin (Life Technologies) in basal medium.

Immunostaining

Primary antibodies used were: 1:1,000 mouse or rabbit anti-TUJ1 (Covance, MMS-435P and PRB-435P), 1:50 rat anti-MBP (Serotec, MCA409S), 1:400 mouse anti-Caspr (UC Davis/NIH NeuroMab Facility, 75-001), 1:400 rabbit anti-PDGFRα (Santa Cruz Biotechnology, sc-338), 1:200 rabbit anti-NG2 (Millipore, AB5320), 1:500 mouse anti-Olig1 (Millipore, MAB5540), 1:500 rabbit anti-Olig2 (Millipore, AB9610), 1:200 rabbit anti-Sox10 (Millipore, AB5727), 1:1000 chicken anti-GFAP (Millipore, AB5541), 1:1000 chicken anti-P0 (Aves Labs, PZO) and 1:500 goat anti-PMP22 (Santa Cruz Biotechnology, sc-18535). For O4 staining, 1:40 mouse anti-O4 (R&D Systems, MAB1326) was added to the medium for 30 min prior to fixation and permeabilization. DAPI (Sigma) was used to visualize nuclei.

Electron microscopy

Oligodendrocytes expressing GFP and neurons expressing RFP were grown on gridded glass-bottomed dishes and fixed as described above. Regions of interest were located and their locations were recorded for future use (supplementary material Fig. S6A).

Next, samples were secondarily fixed overnight in 2.5% glutaraldehyde in PBS. The samples were then fixed in 1% osmium tetroxide/0.3% potassium ferrocyanide in buffer for one hour on ice, washed in water, and *en bloc* stained with 2% aqueous uranyl acetate for one hour on ice. After staining, the samples were dehydrated in a graded ethanol series and embedded in Spurr's resin.

Once the resin was cured, the glass coverslips were removed with hydrofluoric acid and regions of interest were marked by hand and later by the UV beam of a laser capture microdissection microscope (supplementary material Fig. S6A; Zeiss PALM MicroBeam, Jena, Germany). 70-nm cross sections were then taken of the region of interest and imaged on a TEM at 120 KeV (Zeiss Libra 120 PLUS, Oberkochen, Germany)

Dense Wrapping Measurements:

Thick sections were taken and imaged on the TEM as described above for regions that demonstrated dense wrapping. The resulting images were rotated from -90 degrees to +90 degrees in 1 degree steps using a nearest neighbor approximation. A ROI was created at 30 pixels in height (Y-dimension), and a line profile was generated by averaging the pixel intensity values along the y-dimension of the ROI. The raw data from the line profile was smoothed using a low-pass filter with filter coefficients equal to the reciprocal of the span (ie. Moving average).Following this, the lowest four peaks were found using peak analysis.

Array Tomography

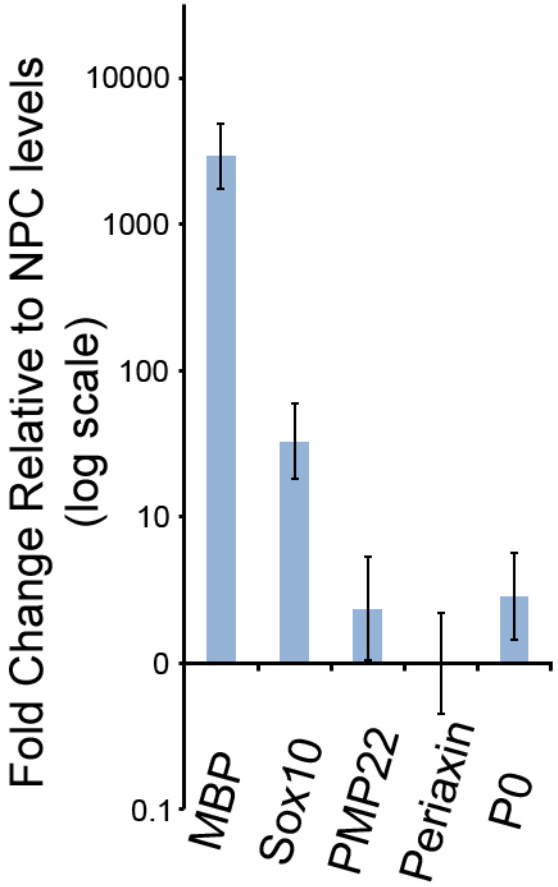
After confirming wrapping events by TEM, 100nm thick serial sections were taken from the same block using a similar device as reported elsewhere (Horstmann et al., 2012), chloroform spread, and placed onto silica wafer chips (Ted Pella, Cat. #16007). The sections were counterstained on the chip with 2% uranyl acetate for 10 minutes followed by 0.4% lead citrate for 4 minutes. Post staining, the chip was subsequently mounted and grounded to an aluminum stub with silver paint. The sections were then imaged in a FE-SEM (Zeiss Sigma VP, Cambridge, UK) at 15 KeV with a 30µm aperture using a 4-quadrant solid-state backscatter detector with the quadrants inverted to produce a TEM-like image. Images were acquired at 12,012 x 6,876 pixels at 2 nm/pixel with a dwell time of 3.5 µs and a line average of 4 using the ATLAS scan engine (Fibics Inc., Ottawa, Canada). Of the 52 serial sections taken, two were omitted due to sectioning and collection artifacts (Slices 12 and 32). The resulting dataset was then stacked and aligned using an affine transform in TrackEM2 (FIJI) and exported to Amira (FEI, Netherlands) for segmentation and 3D rendering. The resulting model was smoothed for appearance purposes by reducing surface details using Amira's built-in smoothing algorithms.

Live imaging

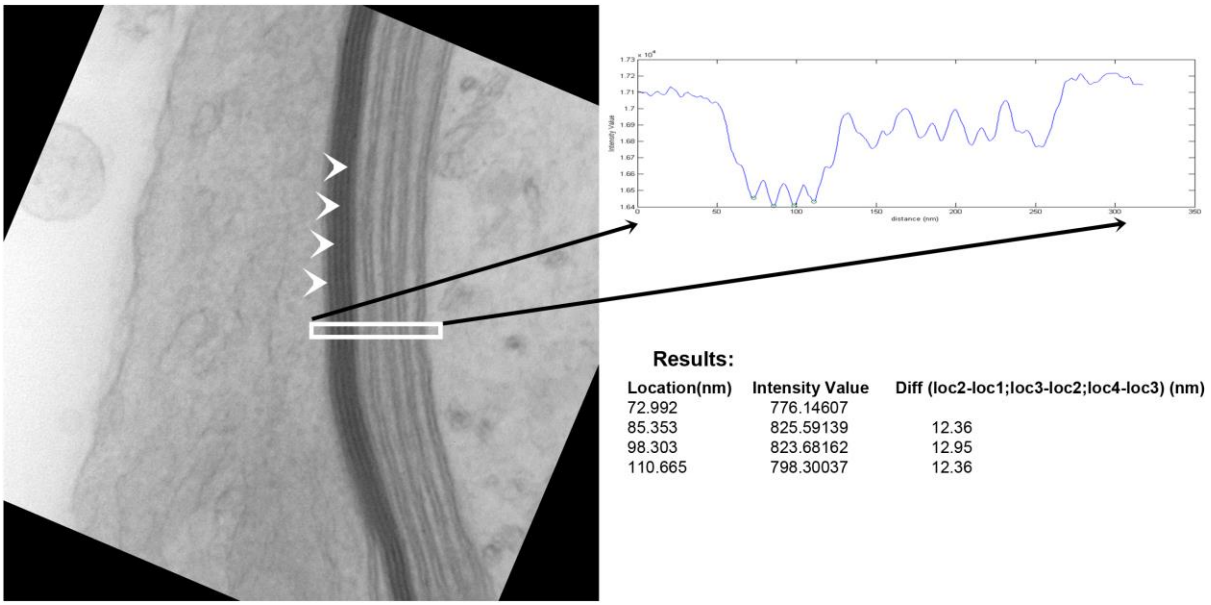
For live imaging, co-cultures were grown either on glass-bottom dishes (GWSt-3522; WillCo Wells) or in microfluidic devices assembled on coverslips. The latter were later attached to a 35-mm plastic dish with a hole in the middle prior to imaging. Neurons were infected with a lentiviral construct expressing either mCherry or membrane-localized TdTomato three to four days prior to initiation of co-culture. Oligodendrocytes were infected with a lentiviral construct expressing membrane-localized EGFP three to four days prior to initiation of co-culture. Imaging was performed on a Zeiss CSU Spinning Disk Confocal Microscope equipped with a stage-top incubation system or on a Yokogawa Cell Voyager 1000 Spinning Disk Confocal Microscope. Optical z-sections were 1 μm apart. Images were taken every 10 min.

Production of viral constructs

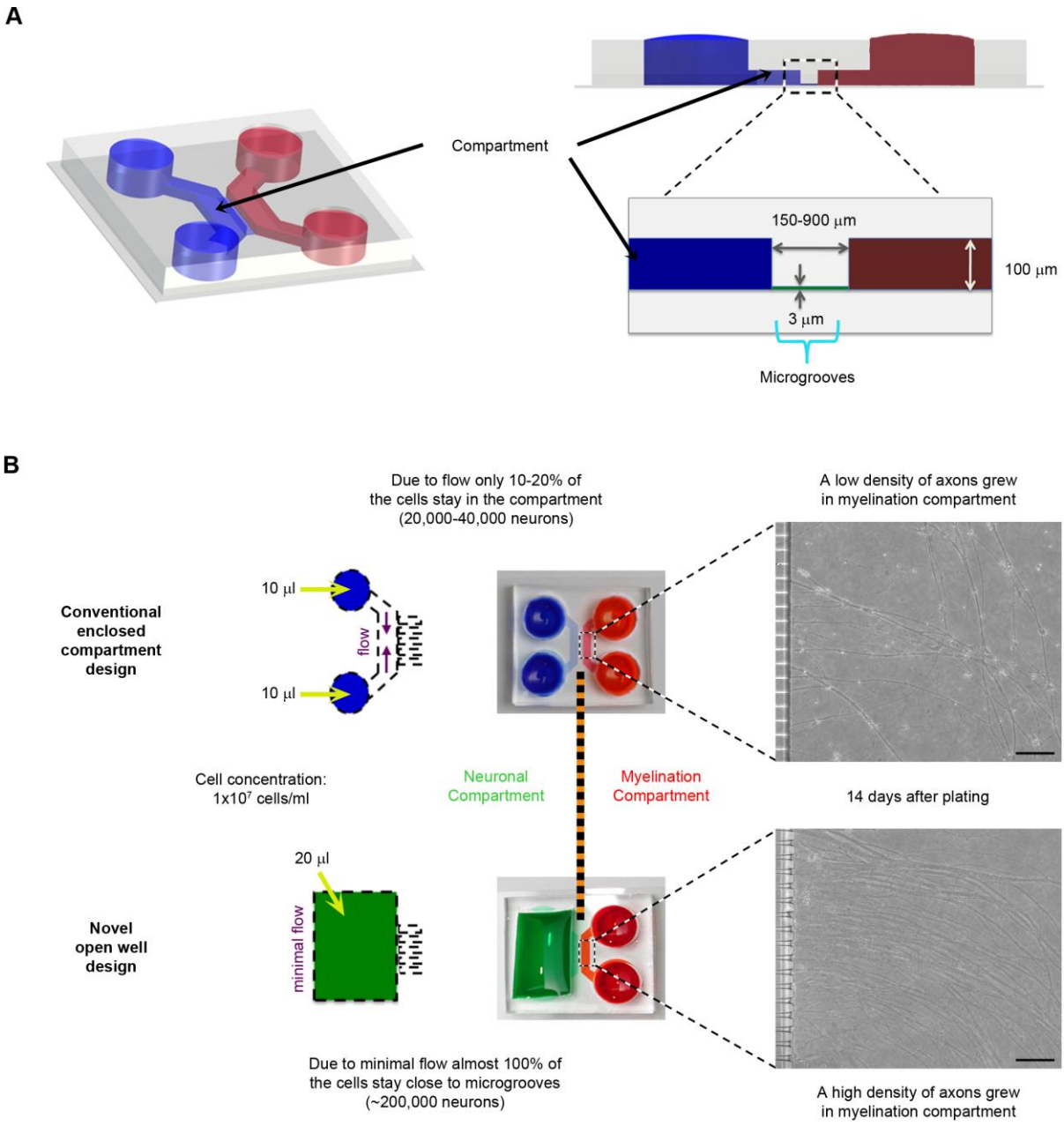
The viral vectors were constructed starting from the plasmid pCSC-Syn-EGFP, which was first modified by insertion of a multiple cloning site, provided by double strand DNA cassette (oligonucleotides G17 and G18), to produce pCSC-Syn-mcs-EGFP. The latter was used to isolate the vectors pCSC-Syn-mcs-mCherry and pCSC-Syn-mcs-tdTomato by replacing the EGFP coding sequence with the PCR amplified mCherry sequence (primers G21 and G22) and tdTomato sequence (primers G23 and G22). pCSC-Syn-mcs-EGFP was also used to derive vector pCSC-MBP-mcs-EGFP, wherein the MBP promoter, obtained by PCR amplification (primers G52 and G53) from the plasmid pMG2-1 (courtesy of A. Gow; Gow et al., 1992), was introduced in place of the Syn promoter. Finally, pCSC-MBP-LckN-EGFP was derived from pCSC-MBP-mcs-EGFP by introducing a double strand DNA cassette (oligonucleotides G54 and G55) encoding the Lck membrane localization domain in the multiple cloning site. pCSC-Syn-LckN-tdTomato was obtained from pCSC-Syn-mcs-tdTomato in a similar way. All constructs include a canonical Kozak sequence and were verified by DNA sequencing. The oligonucleotides used are given in supplementary material Table S3.



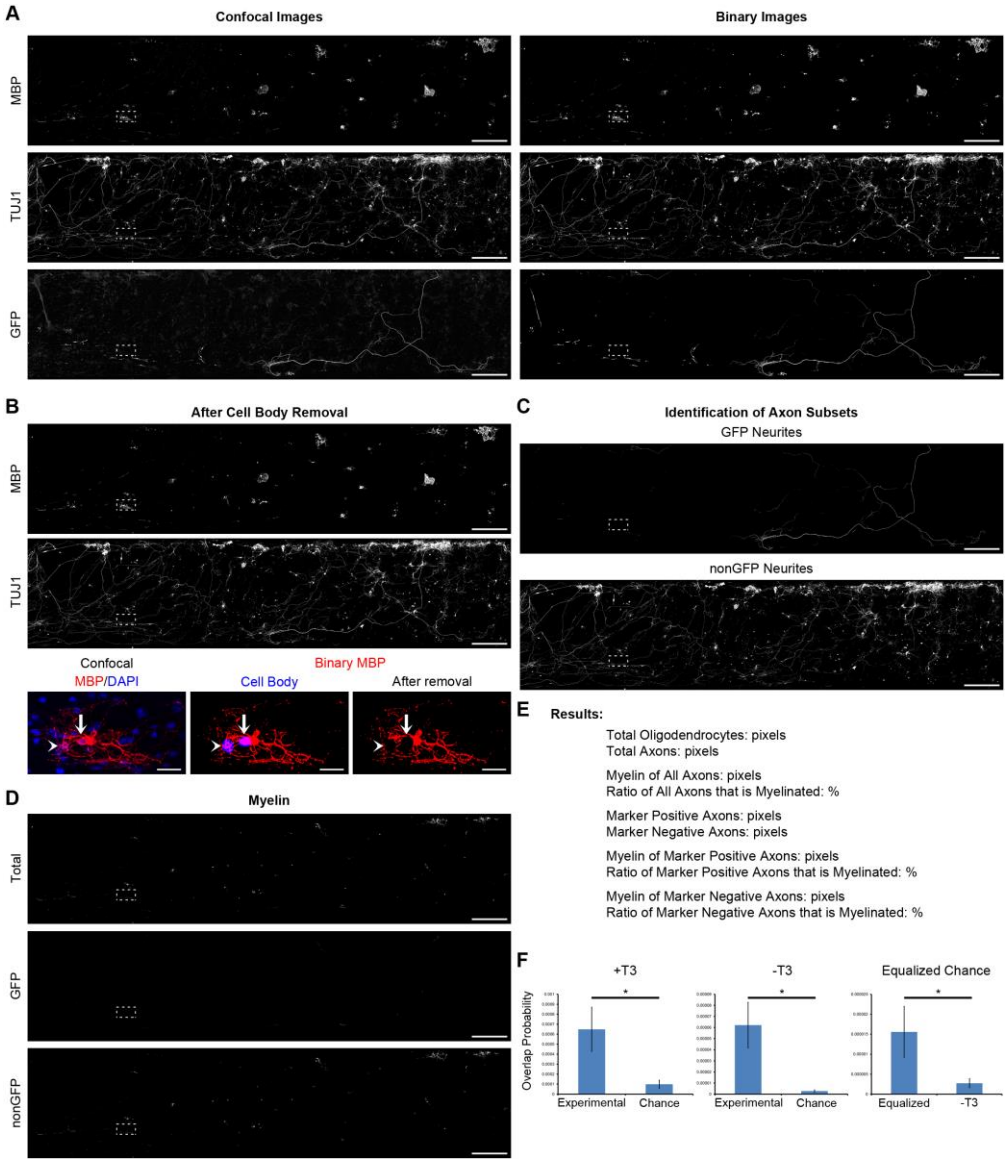
Supplementary Figure S1: Expression level analysis of Schwann cell-specific genes by quantitative-PCR. MBP and Sox10 expression levels are also shown as a reference. Fold changes in expression levels compared to undifferentiated NPCs were plotted in Log-scale. GAPDH expression was used for normalization.



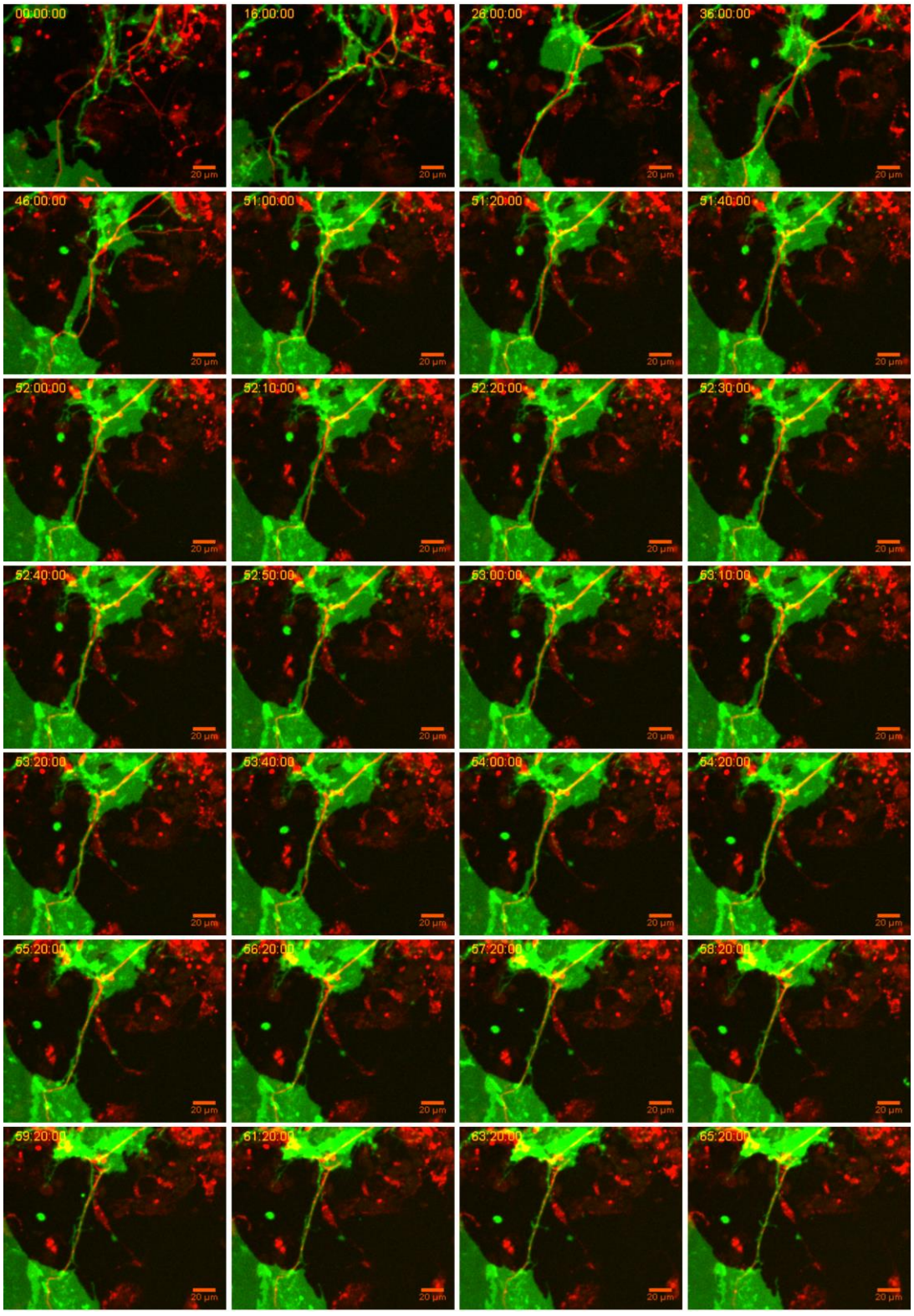
Supplementary Figure S2: Measurement of distance between major dense lines. The intensity of the pixels across compact myelin of an high magnification EM image was measured. The distances between four major dense lines (arrowheads) were shown in nm.



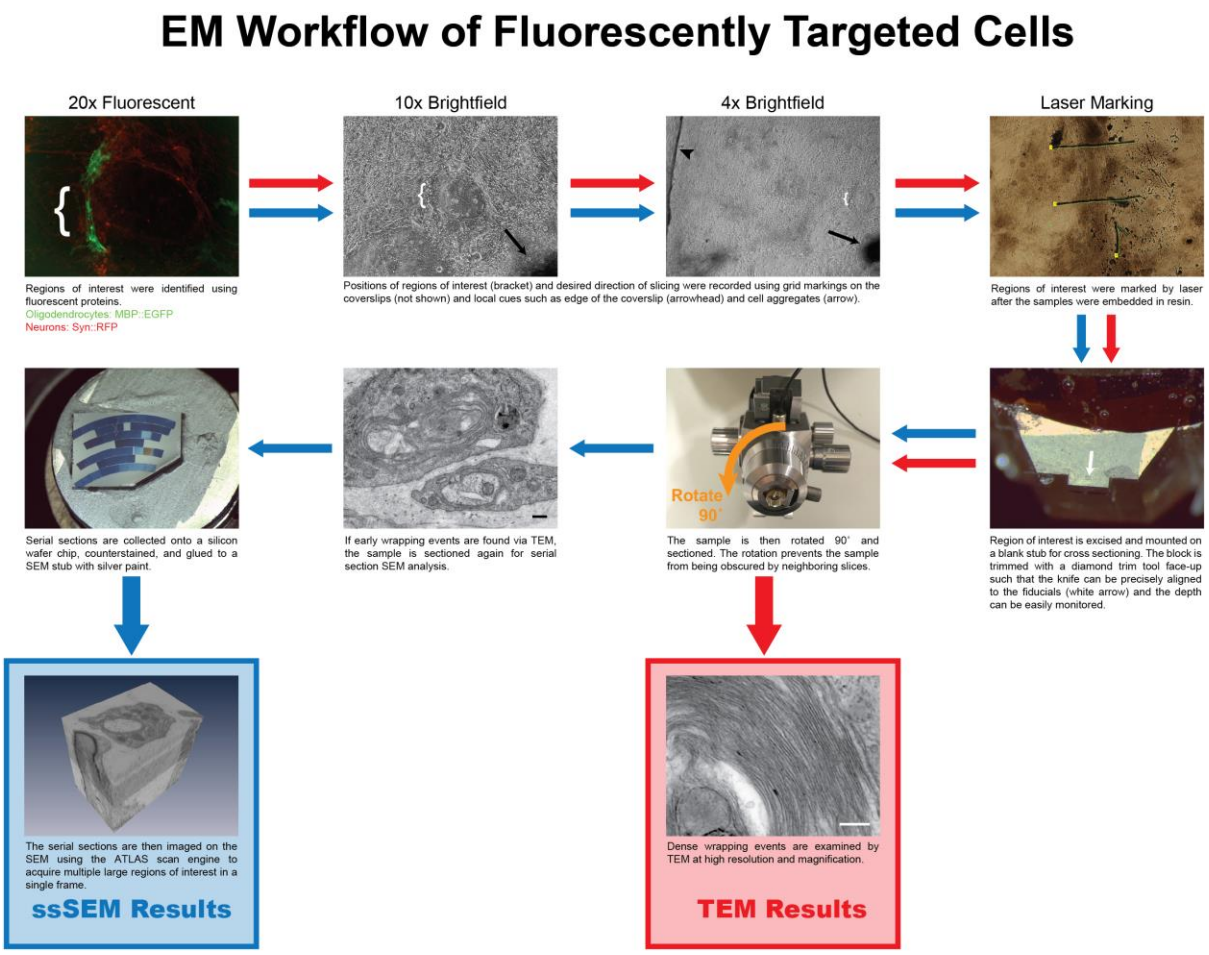
Supplementary Figure S3: Microfluidic devices. (**A**) A 3D model (right) and schematic cross-sections (left) of conventional microfluidic devices. Two enclosed main compartments (blue and red; 100 µm high) are interconnected via microgrooves (3 µm high, 10 µm wide, and 150-900 µm long). (**B**) A comparison of enclosed compartment (upper half) and open well (lower half) microfluidic devices. Left portion shows schematic representations of flow affecting the number of neurons that stay close to microgrooves. Images of actual devices are in the middle. Representative images of axons growing into myelination compartment in two designs are on the right. Scale bars: 150 µm.



Supplementary Figure S4: Quantification of myelin formation. (**A**) Confocal and binary single channel images of merged images in Fig 5A,B. (**B**) Oligodendrocytes (MBP) and neurons (TUJ1) after processing to remove cell bodies. Upper panels: the entire myelination compartment; lower panels: a close-up of the boxed regions in Fig. 5 and S2. Nuclei (blue) identified and grown into cell bodies (blue; arrow). Some nuclei overlap with a large portion of MBP-positive membrane, resulting in false cell body identification (blue; arrowhead). Gaps were left in MBP binary channel after removal (arrow and arrowhead). (**C**) Identified GFP-positive and -negative subsets of axons. (**D**) Identified overlap for the entire myelination compartment of all neurites (Total), of neurites of GFP-expressing neurons (GFP) and of neurites of neurons not expressing GFP (nonGFP). (**E**) CEM output values shown in the panel as pixel counts and percent values. See also the accompanying Users' Guide for CEM. (**F**) Conformation of myelin quantification through comparison between probabilities of experimentally calculated overlap and pure chance overlap (left panels) and equalized probabilities (right panel). See supplementary materials and methods for calculation details. Scale bars: 500 µm.



Supplementary Figure S5: Selected frames from Supplementary Movie 2. The time-frame focuses on a single wrapping event. Note that the oligodendrocyte sending out the two processes that wrap the axon is out of the viewing frame. Scale bars: 20 µm.



Supplementary Figure S6: Electron microscopy workflow of fluorescently tagged cells. The workflow describes how EM data was collected from fluorescently tagged cells. Briefly, regions of interest (ROI) were located via fluorescence and features were noted in brightfield mode at lower magnifications. After EM processing, ROIs were found again using the features noted in brightfield and cutting windows were made using a UV laser. The sample was then trimmed and sectioned either for high-resolution TEM of dense wrapping regions or for serial section SEM (ssSEM) of early wrapping regions. Note: the laser marking and EM images are not from the same dataset but rather were used for demonstration purposes of the procedure. Scale bars: 250nm.

Supplementary Table S1. Percentages and total number of cells counted for assessing differentiation efficiency.

Day 8				
Marker	**Percent total cells**	**Standard error**	**Number of cells expressing the marker**	**Total number of cells**
NG2	37.83	2.44	3201	8,486
PDGF-Rα	37.07	3.54	4007	10,385
Olig1	66.41	1.14	6904	10,385
Olig2	66.56	2.72	6942	10,548
Sox10	85.61	1.37	6431	7,568
O4	2.21	0.24	158	8,469
MBP	0.12	0.04	203	10,385
GFAP	5.09	0.83	402	8,486
TUJ1	9.69	2.13	790	7,568
Day 15				
Marker	**Percent total cells**	**Standard error**	**Number of cells expressing the marker**	**Total number of cells**
NG2	29.19	2.28	1941	6,457
PDGF-Rα	15.69	2.67	885	5,804
Olig1	41.90	4.49	2362	5,804
Olig2	53.48	4.76	4448	8,583
Sox10	63.54	4.78	4981	7,850
O4	13.10	0.79	674	11,236
MBP	4.59	1.31	250	5,804
GFAP	5.08	1.23	337	6,457
TUJ1	8.38	1.69	699	7,850

Supplementary Table S2. Primers used for quantitative PCR analysis.

Gene	Primer pair	Primer sequence
MBP	Forward	TCACACACGAGAACTACCCATT
	Reverse	TGGTGTTCGAGGTGTCACAA
Olig1	Forward	GGTTTCCGAGCTGGATGTTA
	Reverse	GCGAGCCTGAAAAACAGAAC
Olig2	Forward	AGCAATGGGAGCATTTGAAG
	Reverse	CAGGAAGTTCCAGGGATGAA
PLP	Forward	ACCTGGACCACCTGTCAGTC
	Reverse	GAAAGCATTCCATGGGAGAA
MOG	Forward	GCAGGTCTCTGTAGGCCTTG
	Reverse	CCCTCAGGAAGTGAGGATCA
GalC	Forward	CCACTGGACCAACATGACTG
	Reverse	AGCCATTTGCAAAAATCCAG
NG2	Forward	TCCTGGAGAGAGGTGGAAGA
	Reverse	AAGGATGGTGATCGTGAAGG
Sox10	Forward	GACCAGTACCCTCACCTCCA
	Reverse	GGATGGTCCTTTTTGTGCTG
PDGF-Rα	Forward	TGGCATGATGGTCGATTCTA
	Reverse	CGCTGAGGTGGTAGAAGGAG
CNP	Forward	TTCTGAGACCCTCCGAAAAG
	Reverse	CCTTGGGTTCATCTCCAGAA
GFAP	Forward	CACGAACGAGTCCCTAGAGC
	Reverse	GTAGGTGGCGATCTCGATGT
Nestin	Forward	GATCGCTCAGATCCTGGAAG
	Reverse	AGGTGTCTGCAAGCGAGAGT
TUJ1	Forward	GTCTCTAGCCGCGTGAAGTC
	Reverse	GCAGGTCTGAGTCCCCTACA
PMP22	Forward	TTGCTCTTCGTCTCCACCAT
	Reverse	TGGTGAGAGTGAAGAGCTGG
Periaxin	Forward	GACTCACCGGCAGCTAAGAG
	Reverse	GCCCTTCATCTCGTATCCAG
P0	Forward	AGACTACAGTGACAACGGCA
	Reverse	AGAAGAGCAACAGCAGCAAC

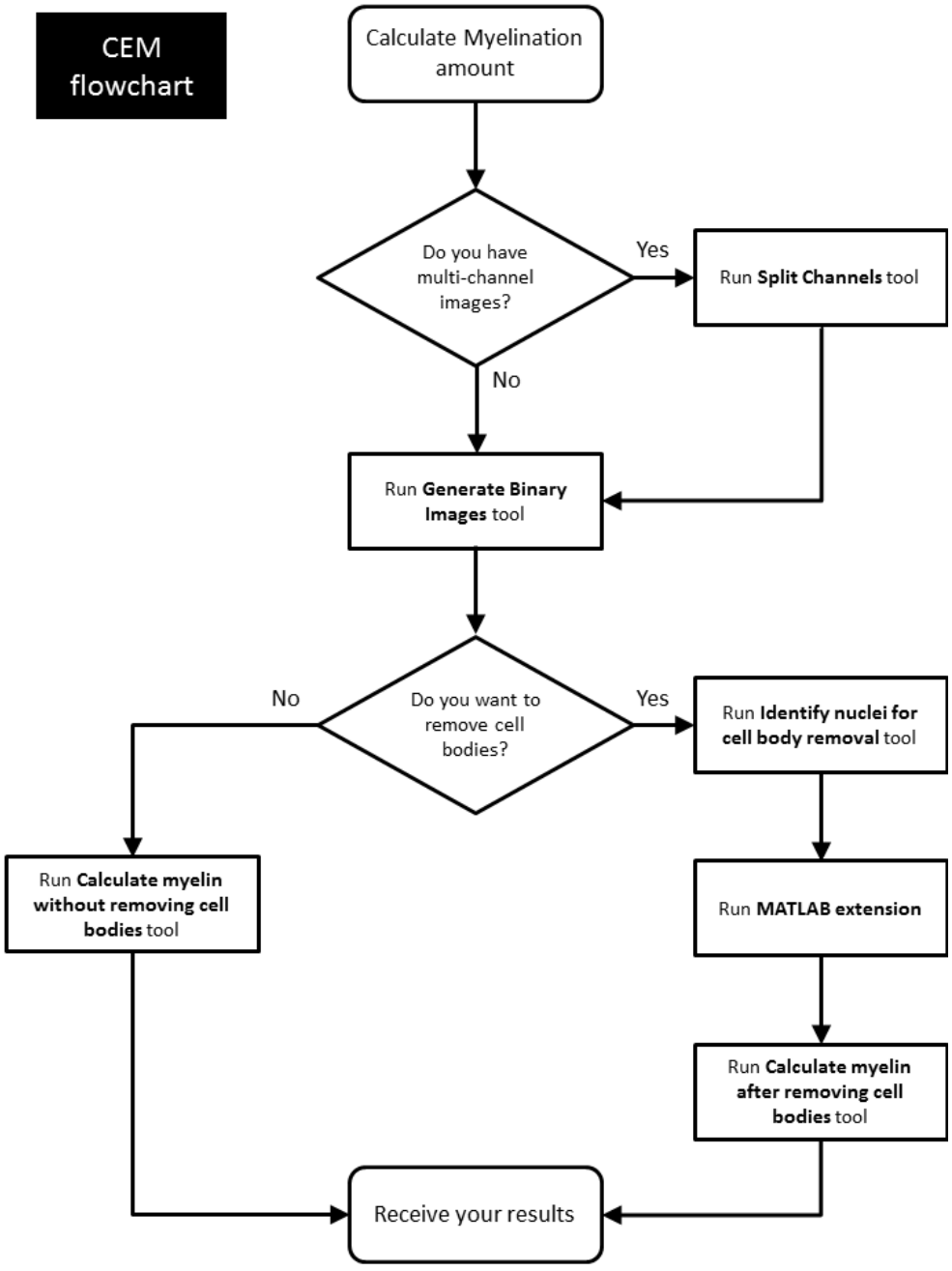
Supplementary Table S3. Oligonucleotides used for reporter plasmid generation.

Oligonucleotide	Sequence
G17	GATCCGGCGCGCCTGCTAGCCTCGAGGGA
G18	CCGGTCCCTCGAGGCTAGCAGGCGCGCCG
G21	TAAAACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGGATA
G22	TACTTGTACAGCTCGTCCATGCCG
G23	TAAAACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGGTCA
G52	TATTATCGATGAGCTCCTTCCTGCTTAGGCCG
G53	GCGGGGATCCTAGAATTATTCGAGCTT
G54	CGCGCCACCATGGGCTGTGGCTGCAGCTCACACCCGGAAGATGGA
G55	CCGGTCCATCTTCCGGGTGTGAGCTGCAGCCACAGCCCATGGTGG

Appendix S1: Users' Guide to CEM

This document explains how to use Computer-assisted Evaluation of Myelination (CEM), which was developed by Bilal E. Kerman and Krishnan Padmanabhan under Fred H. Gage's guidance at the Salk Institute in La Jolla, CA, USA. The ImageJ code is written by B.E.K. and MATLAB code is written by K.P. CEM is provided as part of the fair use license. Please reference Kerman et al. (submitted).

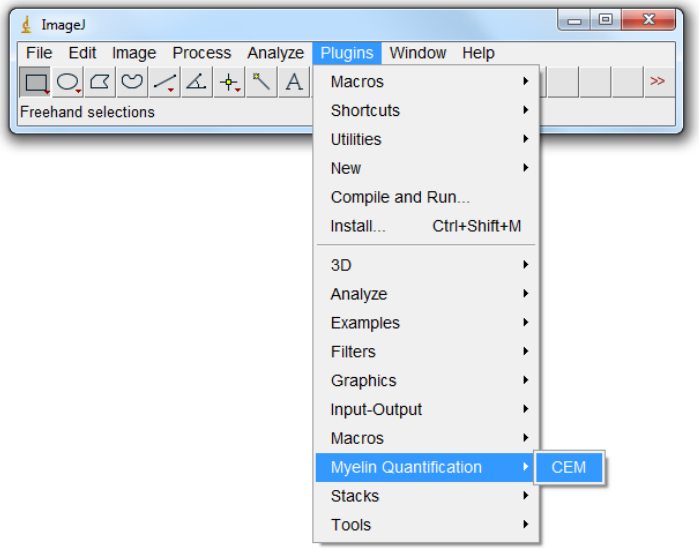
CEM is dedicated to the loving memories of Cem and Dilay Kerman, who were taken from us far too early.



You need an ImageJ version 1.47i or higher to use the calculator. If you want to use the "remove cell bodies" function, you need to run the accompanying MATLAB Toolbox that was tested on MATLAB 2012b. The flowchart above gives a quick look at how the calculator operates and can be used as a quick reference while running it.

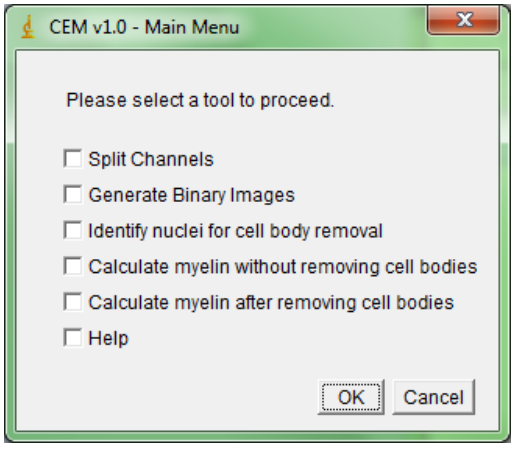
How to RUN?

You can run CEM either by first copying the file under the ImageJ plugins folder and starting it from the menu, as seen in the figure below, or by directly opening the "CEM.ijm" file in ImageJ and select "RUN" in the menu option. MATLAB Toolbox can be run by opening the file in MATLAB (see below). If you are processing large image files (>500mb), we recommend a computer with a fast hard-drive for quicker read and write times.



The main menu:

When you start CEM, you will be greeted by a welcome screen. Then you'll get to the main menu. Here you can check on the operation you want to perform.

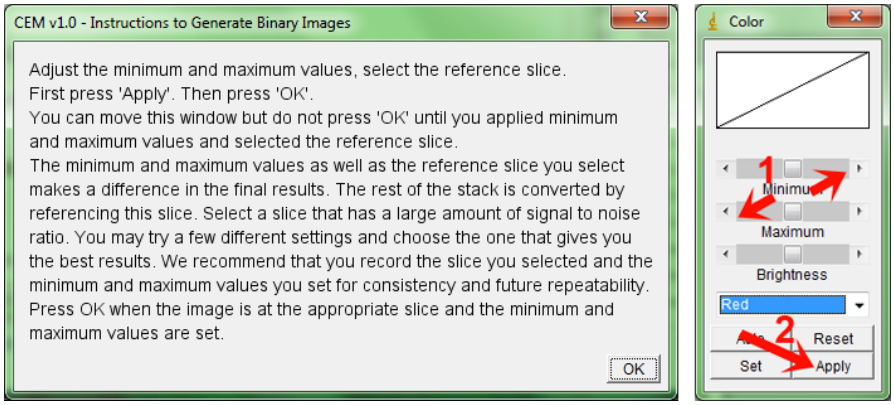


Split channels:

If your images are multi-channel images such as lsm files, composite Tiffs or RGB files, you have to split them into single channel images. The 'Split Channels' tool is designed for that purpose. It will accept any RGB or composite file that ImageJ can open and will ask you to save the new images.

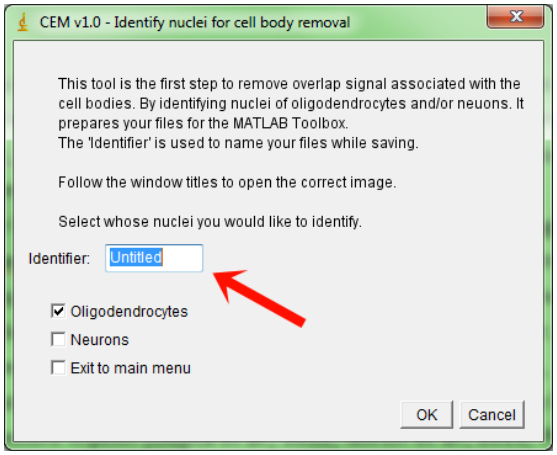
Generate binary images:

The latter calculations are performed on binary images only. This tool will take your single channel images and convert them into binary. As seen in the figure below, you will be asked to adjust brightness by setting minimum and maximum values for your image and select a reference slice. It is crucial that you set brightness to maximize signal-to-noise ratio (1) and press “Apply” (2). If you have image stacks, you need to bring your stack to the reference slice before pressing “OK.” The reference slice is used by the ImageJ binary conversion algorithm to determine values for the rest of the stack. Therefore, it is crucial to select a slice with a large amount of positive signal and low background noise. We suggest that you try a few different settings until you identify the optimum ones and that you record your settings.

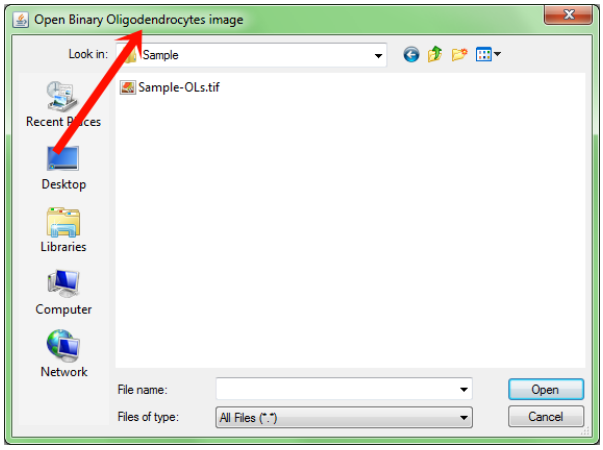


Identify nuclei for cell body removal:

This tool helps you to remove overlap signal associated with the cell bodies. This is a two-step process. First, you need to find the nuclei of the cells you are interested in using this tool. The input files are binary images of oligodendrocytes and/or neurons and nuclei (outputs of “Generate Binary Images” tool or generated by other sources). The first window will ask you to enter an “Identifier” that is used for naming your images and to make a selection as seen in the figure below. The “Identifier” can be any combination of letters, numbers and symbols that are allowed in a file name such as “Exp1-Image1.”



Next you will be asked to choose the image to open. Please follow the window labels to open the correct image as seen in the figure below.

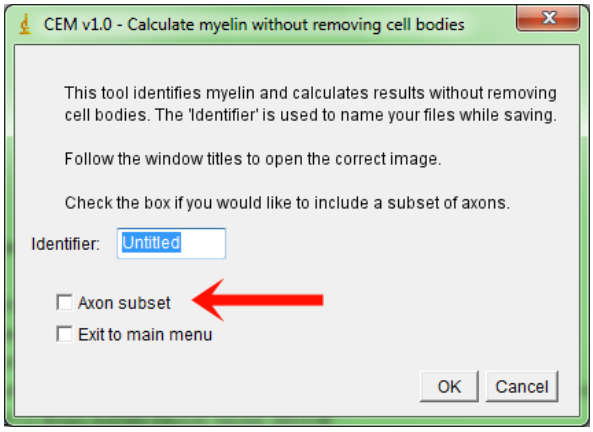


Once the nuclei are identified, the calculator will save the resulting images in a folder that you choose or in the same folder if you just press save.

Second, you need to remove the noise and grow the nuclei into cell bodies using the accompanying MATLAB Toolbox (see below). The application removes particles smaller than a preferred pixel area (for example, 50 pixels square) and grows the remaining nuclei into cell bodies by a preferred number of pixels (for example, 5 pixels).

Calculate myelin without removing cell bodies:

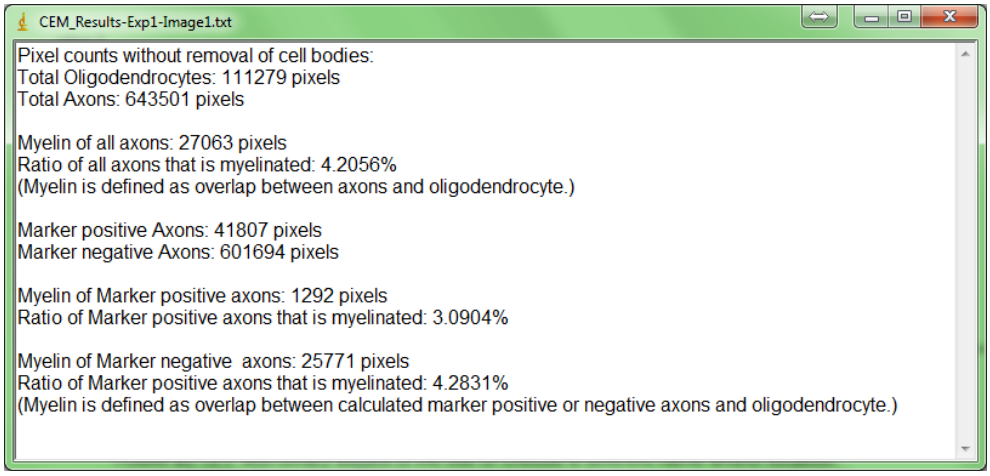
This is the tool where the myelin is identified (as the overlap between axon and oligodendrocyte images) and the results are generated. The input files are binary images of oligodendrocytes and neurons (outputs of “Generate Binary Images” tool or generated by other sources). The first window will ask you to enter an “Identifier” that is used for naming your images (see above) and to select if a subset of axons is different than the others, as seen in the figure below.



Checking the "Axon subset" option allows you to distinguish some of your axons from the others. For example, a subset of your neurons may be expressing a shRNA to knock down a gene that you suspect affects the myelination of these neurons. As long as the subset of axons is also visually distinct, for example via GFP expression, the calculator will identify both marker-positive and -negative axons. You will need a separate binary image of the distinguishing marker such as GFP.

Next you will be asked to choose the images to open. Please follow the window labels to open the correct images, as explained above.

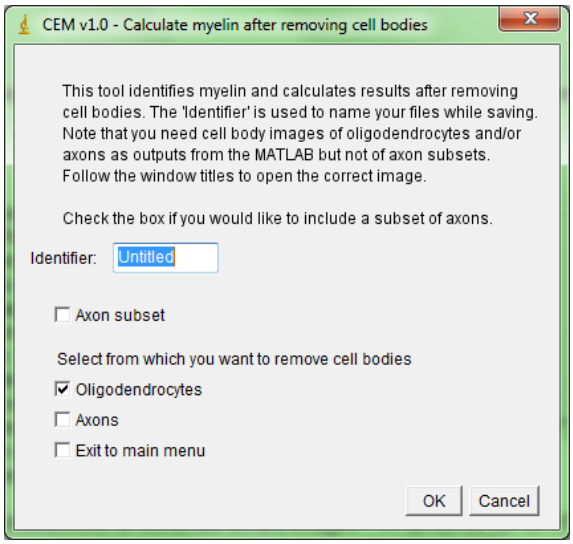
Once the images are opened, the calculator will start to identify myelin. The myelin is identified as overlap between the oligodendrocyte and axon images by using the AND operator on binary images. The process may take a few minutes, depending on the size of your images and the specifications of your computer. During this process, new images will be generated. When the calculations are complete, the results will be displayed in a new window as seen below, and you will be asked to save the file as a text document. You will also be asked if you want to save the generated images of myelin and subsets of axons. The results are generated as pixel counts and percent values. The pixels are counted using the "Histogram" function of ImageJ (on maximum intensity projections if images are stacks).



Calculate myelin after removing cell bodies:

This tool is practically the same as the "Calculate myelin without removing cell bodies" tool but removes the cell bodies before identifying myelin. The input files are binary images of oligodendrocytes and neurons (outputs of "Generate Binary Images" tool or generated by other sources) and cell body images as the combined output of "Identify nuclei for cell body removal" and MATLAB Toolbox.

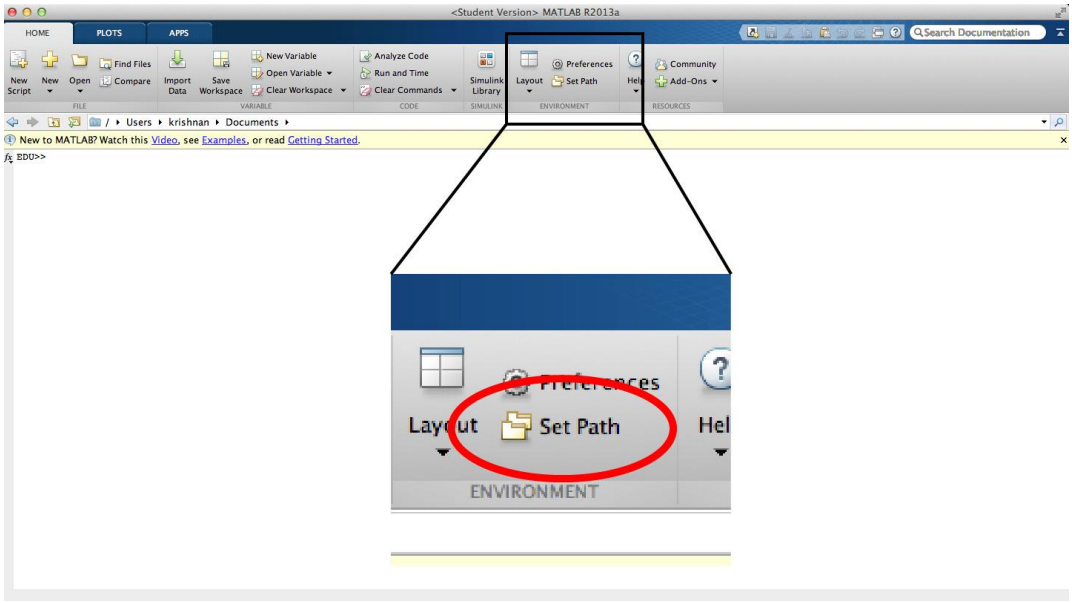
The "Identifier" and "Axons subset" work as described above. You need to check if you want to remove cell bodies from oligodendrocytes and/or neurons. Do not forget to follow the window labels to open the correct image. The output is the same as "Calculate myelin without removing cell bodies" but "CBR" will be added to the titles of any output files to distinguish them.



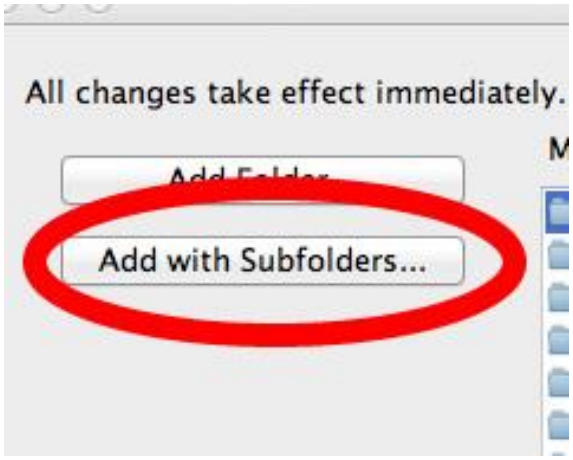
MATLAB Toolbox Installation and Setup:

The MATLAB CEM Toolbox was developed in MATLAB Version R2013a is the OS X 10.9.4 and has been tested on a Windows 7 computer in MATLAB Version R2012b. **NOTE: The INSTALLATION AND SETUP needs to be done ONLY ONCE when the application is FIRST installed on a COMPUTER. After setting up the software, the user can jump to the instructions for running the application.**

Following download of the CEM package, all files should be copied to a directory of the user's choosing. After launching MATLAB, click on Set Path under the Environment.

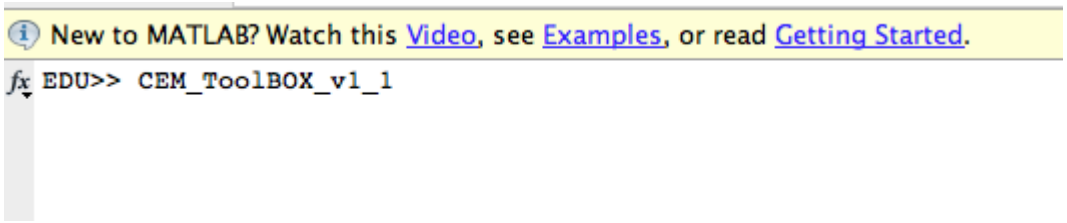


Select Add with subfolders and choose the folder where the downloaded files were copied. Following this, select SAVE and the CLOSE.



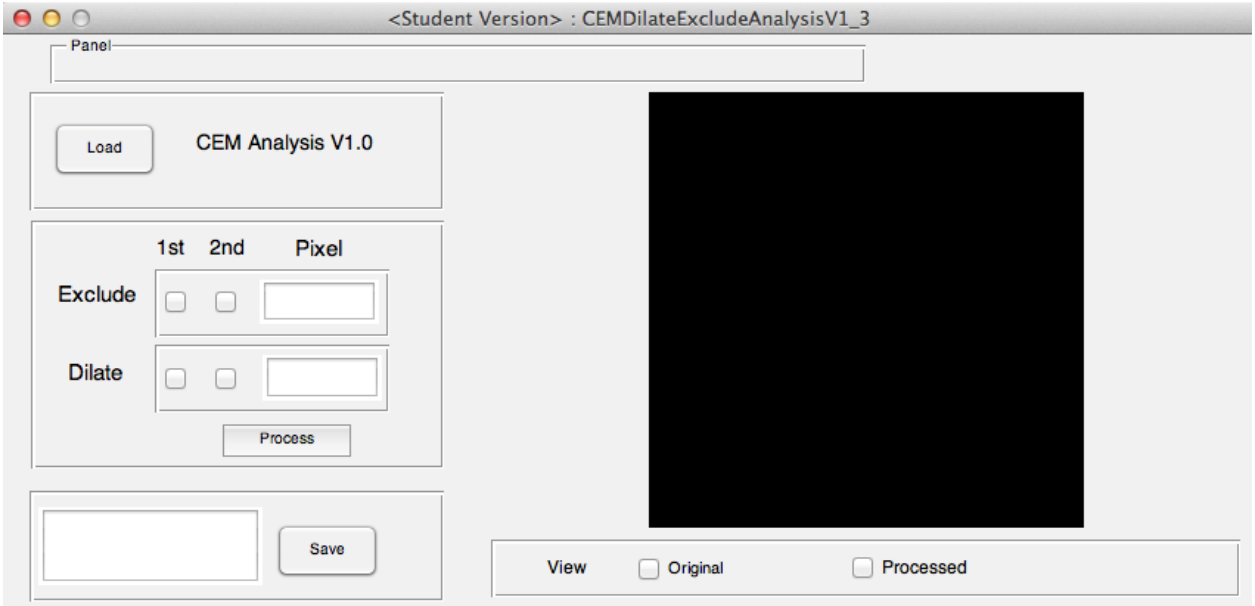
MATLAB toolbox processing:

Following installation, CEM is launched by typing CEM_ToolBOX_v1_1 in the command line of MATLAB.

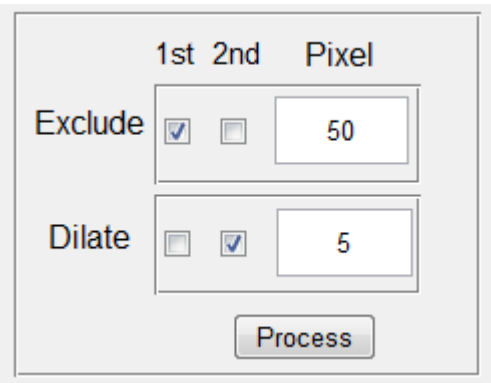


This toolbox allows the user to perform the exclude and dilate functions described in the manuscript. First, a file is loaded by clicking on the LOAD button. The CEM toolbox processes the following file formats (RGB tif, grayscale tif, grayscale tif stack, RGB and grayscale jpeg). Once the file has loaded, it will appear as an image in the image window, and

the VIEW original toggle box will be checked. If the file is a grayscale image or RGB image, it will appear as such. If the file is a tif stack, it will appear as a maximum intensity Z projection in the viewer.



The user has the ability to select different combinations of operations include, exclude only, dilate one, exclude first, then dilate, dilate first, then exclude. This can be done so by toggling between the different conditions in the process toolbox on the left.



In the example above, CEM will perform object exclusion on the image first and then dilate the image. In this case, the algorithm will exclude objects less than 50 pixels in size, and then dilate the resultant image 5 pixels. This allows for maximum flexibility of parameters for processing. Once the desired operations are selected, click PROCESS.

Depending on the size of the image, this may take some time (and a status bar will note this). Once complete, the image will be displayed in the image window, and the VIEW Toggle box will shift to Processed. The user can toggle between the two images by selecting the Original or Processed toggle box. If a change in the processing parameters is desired, this can be achieved, and the PROCESS button should be clicked. The code will only save the most recent Processed Image.

Once the desired parameters are achieved, and the user is happy with the processing, the image can be saved by selecting the SAVE button. **IMAGES WILL NOT BE SAVED UNLESS THE SAVE BUTTON IS EXPLICITLY CLICKED.** The user may select a name for the file to be saved as (the format the file will be saved is a tif or tif stack, matching the format of the original image). If no file name is entered in the save text box, then a default file name will be used modifying the original file name with the addition of _Processed in the name.

MATLAB Toolbox Exiting and Quitting:

BEFORE QUITTING, ENSURE THAT IMAGES ARE SAVED, AS THIS IS NOT DONE UNLESS THE SAVE BUTTON IS EXPLICITLY CLICKED. The user may quit the program by simply closing MATLAB.

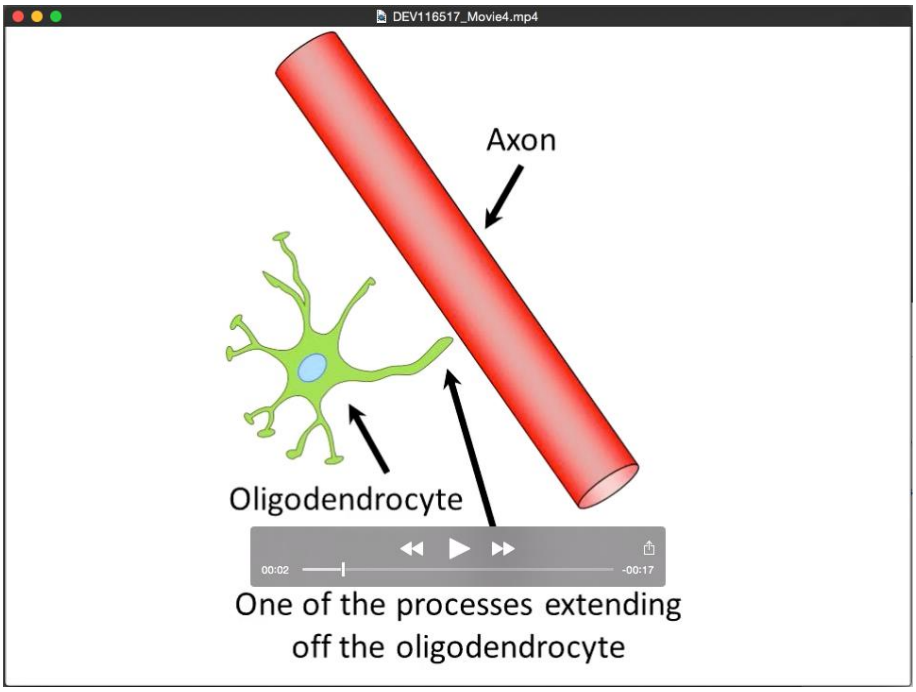
Click here to Download Appendix S2-CEM package

SUPPLEMENTARY MOVIES

Movie 1: An oligodendrocyte wrapping axons monitored in real time for almost eight days. Arrows point to anchor points and oligodendrocyte processes in the course of wrapping. Images were taken every 10 min and are shown here at 12 frames per second. Each frame is a maximum intensity projection of 1 µm apart optical sections. Time is shown as hours:minutes.

Movie 2: A single wrapping event was monitored in real time focusing on the oligodendrocyte processes. The oligodendrocyte sending out the processes is out of the frame at the top. Images were taken every 10 min and are shown here at 12 frames per second. Each frame is a maximum intensity projection of 1 µm apart optical sections. Time is shown as hours:minutes.

Movie 3: 3D reconstruction of serial EM sections were animated to visualize the wrapping from every angle.



Movie 4: An animation of SARAPE model of myelination.