Bioimage informatics

MyelinJ: an ImageJ macro for high throughput analysis of myelinating cultures

Michael J. Whitehead, George A. McCanney, Hugh J. Willison and Susan C. Barnett*

Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8TA, UK

*To whom correspondence should be addressed.

Abstract

Summary: MyelinJ is a free user friendly ImageJ macro for high throughput analysis of fluorescent micrographs such as 2D-myelinating cultures and statistical analysis using R. MyelinJ can analyse single images or complex experiments with multiple conditions, where the ggpubr package in R is automatically used for statistical analysis and the production of publication quality graphs. The main outputs are percentage (%) neurite density and % myelination. % neurite density is calculated using the normalize local contrast algorithm, followed by thresholding, to adjust for differences in intensity. For % myelination the myelin sheaths are selected using the Frangi vesselness algorithm, in conjunction with a grey scale morphology filter and the removal of cell bodies using a high intensity mask. MyelinJ uses a simple graphical user interface and user name system for reproducibility and sharing that will be useful to the wider scientific community that study 2D-myelination in vitro.

Availability and implementation: MyelinJ is freely available at https://github.com/BarnettLab/MyelinJ. For statistical analysis the freely available R and the ggpubr package are also required. MyelinJ has a user guide (Supplementary Material) and has been tested on both Windows (Windows 10) and Mac (High Sierra) operating systems.

Contact: Susan.Barnett@glasgow.ac.uk

Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

Myelin is an essential component of the central nervous system (CNS) and its degeneration is associated with spinal cord injury and several CNS diseases, most notably multiple sclerosis (Goldenberg, 2012). Myelinating cultures generated from dissociated embryonic rodent spinal cords have been developed (Kerman et al., 2015; Pang et al., 2012; Sorensen et al., 2008; Thomson et al., 2008) as a tool to study developmental myelination (Ioannidou et al., 2012), characteristics of spinal cord injury (Boomkamp et al., 2012) and demyelination (Lindner and Linington, 2016). Consequently, myelinating cultures can be used as a high throughput screen for potential therapeutics that promote (re)myelination (McCanney et al., 2018, 2019). One of the main bottlenecks for these screens is the accurate high throughput quantification of myelin sheaths. Currently, only a CellProfiler pipeline is freely available for analysis of in vitro myelination (available at https://github.com/mues/cop). The MyelinJ ImageJ plugin we have developed is a freely available ImageJ (Schindelin et al., 2012) macro that allows for high throughput analysis of individual experiments or large studies. The macro produces the percentage (%) of myelination and the % of neurite density for each image and links to R (R Core Team, 2013) for automated statistical analysis and graph production. The user friendly graphical
user interface (GUI) and username system support reproducibility and sharing. MyelinJ aims to be widely applicable to the neuroscience community, because the settings can be easily adjusted/optimized specifically for your experiment. This study has only tested MyelinJ using myelinating cultures, however it is likely to also be useful for analysing slice cultures (Hill et al., 2014) and tissue sections.

2 Materials and methods

Myelinating cultures were made according to Sorensen et al. (2008), demyelinated as described in McCanney et al. (2019) and manual analysis of micrographs was performed according to Sorensen et al. (2008). MyelinJ analyses % myelination and % neurite density of 2D fluorescent micrographs.

2.1 Background subtraction

Background is first subtracted either using ImageJ’s ‘rolling ball’ background subtraction, or using the neurite image as a mask to remove any bleed through of neurite fluorescence, followed by subtraction of pixels below a user provided threshold (the user can also select no background subtraction).

2.2 Myelin sheath selection

The Frangi vesselness (Frangi et al., 1998) plugin is used to select myelin sheaths. Non-myelin sheath pixels are removed using a combination of ‘grey scale attribute filtering’ from the MorphoLibJ library (Legland et al., 2016) and removal of a high intensity mask (that corresponds to cell bodies); both of which are optional. % myelination is calculated as:

\[
\text{Total myelin pixels/Total neurite pixels} \times 100.
\]

2.3 Neurite selection

The percentage of (%) neurite density is calculated using the ImageJ filter ‘normalize local contrast’ (NLC). Alternatively, all standard ImageJ thresholding methods are also available to the user.

2.4 Neurite density analysis

% neurite density is calculated as:

\[
\text{Total neurite pixels/Total pixels} \times 100.
\]

2.5 Statistical analysis

MyelinJ links ImageJ to R via the command line and uses the ggpubr package (https://github.com/kassambara/ggpubr) for statistical analysis and the production of publication ready graphs. MyelinJ performs Welch’s T test followed by correction for multiple testing using the false discovery rate (FDR). The user can choose between comparing all experimental conditions to each other or comparing all experimental conditions to control only.

3 Results

MyelinJ calculates ‘% myelination’ and ‘% neurite density’. In order to test the macro we first analysed an in vitro myelinating time course and compared it to the freely available CellProfiler pipeline (Fig. 1A–E, Supplementary Fig. S1 illustrates comparisons for the entire time course). MyelinJ is able to identify about 46% more myelin sheath pixels on average per image than CellProfiler, based on a comparison of 30 images at each time point taken from three technical replicates. In previously published data based on this
model this is the standard number of images to constitute a biologic-
el replicates (Fig. 1E and F and Supplementary Fig. S1C). MyelinJ’s
neurite density is also more consistent across images (Fig. 1J–M and
Supplementary Fig. S4). MyelinJ was also compared to manual anal-
ysis of % myelination by drawing over the myelin sheaths (Fig. 1G–
I). MyelinJ is very similar to manual analysis and takes ~15–18 s per
image compared to about 15 min per image. Furthermore, we find
that Frangi vesselness is superior to the Otsu thresholding method in
ImageJ for determining % myelination, as illustrated in
Supplementary Figure S1E.

MyelinJ’s ability to ignore non-myelin sheath background was
tested using myelinating cultures that have been demyelinated in a
complement-mediated manner (McCanney et al., 2019). Demyelin-
ation leaves significant background, which is predominantly not myelin sheaths (Fig. 1N). MyelinJ effectively disregards the non-myelin sheath background (Fig. 1M). In comparison, CellProfiler misses the majority of these myelin sheaths (for Fig. 1N CellProfiler identified 0% myelination—
image post analysis not shown). Supplementary Figure S2 illustrates
the graphs and statistical analysis performed by MyelinJ for an ex-
ample dataset (demyelination versus remyelination), using the
ggpubr package in R.

4 Conclusions

This newly developed MyelinJ is a user friendly ImageJ macro for the
analysis of fluorescent micrographs of 2D myelinating cultures
providing quantification of the % of neurite density and the % of
myelination. To the best of our knowledge, there are currently no
other publicly available ImageJ macros for this analysis and MyelinJ
marks a significant improvement upon the freely available
CellProfiler pipeline (available at https://github.com/mucos/cp),
being able to more accurately analyse both myelin sheaths and neu-
rites. MyelinJ also offers automated calculation of % neurite density and % myelination in order to avoid human error, can analyse com-
plex experiments where a summary of the results for each condition is
provided and seamlessly links to R for the graphical representa-
tion of results and statistical analysis. In addition, MyelinJ analyses
myelin sheaths significantly better than the Otsu ImageJ threshold-
ing algorithm (Otsu was selected following comparisons between all
ImageJ thresholding algorithms). This is the first time (to the best of
our knowledge) that an ImageJ macro that can interact with the stat-
estistical package R has been made freely available. MyelinJ uses the
ggpubr package to perform statistical analysis and produce publica-
tion quality graphs, providing a seamless analysis pipeline from raw
images to graphical representation and statistical analysis for high
throughput screens.

Author contributions

M.J.W. conceived the study, wrote the main manuscript text and
prepared all figures. G.M. performed all of the rat myelinating spi-
nal cord culture experiments. S.C.B. and H.J.W. directed the study
and wrote the manuscript. All authors reviewed, edited and
approved the final manuscript.

Funding

This work was supported by a Doctoral Training Grant to M.J.W. provided
jointly by the UK MRC [MR/K501335/1] and UK BBSRC [BB/J013854/1],
the Wellcome Trust [202789/Z/16/Z], Medical Research Scotland (MRS;
G.M.) and MS society of Great Britain (56).

Conflict of Interest: none declared.

References

Boomkamp, S.D. et al. (2012) The development of a rat in vitro model of spinal
cord injury demonstrating the additive effects of Rho and ROCK inhibitors
on neurite outgrowth and myelination. Glia, 60, 441–456.

Frangi, A.F. et al. (1998) Multiscale vessel enhancement filtering Medical
Image Computing and Computer-Assisted Intervention—MICCAI’98.
Wells, W.M. et al. (eds) Lecture Notes in Computer Science. Multiscale
Vessel Enhancement Filtering. Springer, Berlin, Heidelberg, pp. 130–137.

Goldenberg, M.M. (2012) Multiple sclerosis review. Pharm. Therap., 37,
175–184.

Hill, R.A. et al. (2014) Organotypic slice cultures to study oligodendrocyte dy-
namics and myelination. J. Visual Exp., 90, e51835.

Ioannidou, K. et al. (2012) Time-lapse imaging of the dynamics of CNS
glial-axonal interactions in vitro and ex vivo. PLoS One, 7, e30775.

Kerman, B.E. et al. (2015) In vitro myelin formation using embryonic stem
cells. Development, 142, 2213–2225.

Legland, D. et al. (2016) Morphololibj: integrated library and plugins for math-
ematical morphology with ImageJ. Bioinformatics, 32, 3532–3534.

Lindner, M. and Lintong, C. (2016) Myelinating cultures: an in vitro tool to
identify demyelinating and axopathic autoantibodies. Methods Mol. Biol.,
1304, 105–114.

McCanney, G.A. et al. (2018) Neural cell cultures to study spinal cord injury.
Drug Discovery Today, 25-26C, 11–20.

McCanney, G.A. et al. (2019) Low sulfated heparins target multiple proteins
for central nervous system repair. Glia, 67, 668–687.

Pang, Y. et al. (2012) Neuron-oligodendrocyte myelination co-culture derived
from embryonic rat spinal cord and cerebral cortex. Brain Behav., 2, 53–67.

R Core Team (2013) A language and environment for statistical computing.

Schindelin, J. et al. (2012) Fiji: an open-source platform for biological-image
analysis. Nat. Methods, 9, 676–682.

Sorensen, A. et al. (2018) Astrocytes, but not olfactory ensheathing cells or
Schwann cells, promote myelination of CNS axons in vitro. Glia, 56, 750–763.

Thomson, C.E. et al. (2008) Myelinated, synapsing cultures of murine spinal
cord—validation as an in vitro model of the central nervous system. Eur. J.
Neurosci., 28, 1518–1535.