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Automated ImageJ/FIJI plug-in for morphological study of neurons and biological objects (LUSCA)

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Introduction: The plasticity of the mammalian brain is achieved through fine-tuning of the number of contacts among cells. This means that the cell's length, shape, and the quantity of its projections are not static parameters. As such, any image analysis is faced with the quantification of thousands of cells of various sizes, shapes, and dimensions, requiring specialized, often proprietary programs.

Aim: The main goal was to develop a new plug-in for ImageJ/Fiji that can perform automatic morphological analysis of neurons.

Materials & methods: Neural stem cells, isolated from the telencephalic wall of 14.5 days old mouse embryos, were cultivated in differentiation medium for ten days, and labelled with MAP2 and SMI312. Images were analysed with Lusca, validated against the golden standard (manual analysis), and compared to similar scripts: NeuronJ, NeuriteTracer, NeurphologyJ, CellProfiler and Imaris. The unpaired t-test was used for the statistical analysis of width, while all the other analysis was done using the one-way ANOVA with the Tukey Kramer multiple comparisons post-hoc test.

Results: Lusca performs image segmentation using a machine learning process - Trainable Weka Segmentation, followed by fine tuning with respect to intensity and size thresholds. Particle analysis is used for determination of area/volume, number, and intensity of the objects, while the length and width analysis are performed using one-pixel lines and Local Thickness. The colocalization analysis is based on the JaCoP plug-in. Comparison with similar programs revealed that Lusca offers more options for analysis, with faster and more accurate quantifications, including better noise elimination and recognition of barely visible details.

Conclusion: Lusca is a useful tool for automatic, fast, and accurate analysis of images of neurons, representing a viable and time-saving alternative to current open-source software. Since it utilizes machine learning, it also facilitates analysis of other biological objects such as mitochondria and blood vessels.

Keywords: automated quantification, image analysis, ImageJ, machine learning, neuronal morphology