Image Analysis of Neurite Branching: High-Content Screening at High Speed

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Automated image analysis is becoming a central tool for quantifying the effect of candidate drugs on cells. A number of commercial high-content cellular imaging systems include software that can characterize neuronal projections (i.e., axons and dendrites). The faster systems, capable of running at primary screening speeds, tend to measure only the simplest feature describing these neurite outgrowths—their total length. The slower systems, suitable for secondary screening, are given a greater amount of time to run more sophisticated algorithms. Some of these can measure an additional feature describing neurite complexity—the number of branch points (*Figure 1*).

This paper presents a powerful software tool (CSIRO Mathematical and Information Sciences, North Ryde, Australia) that can simultaneously report several features to characterize the branching topology of each neurite "tree." The software can discriminate subtle changes in neurite branching structures that would otherwise go undetected. The code is adapted for primary screening and can also run as a standalone system for fundamental research.

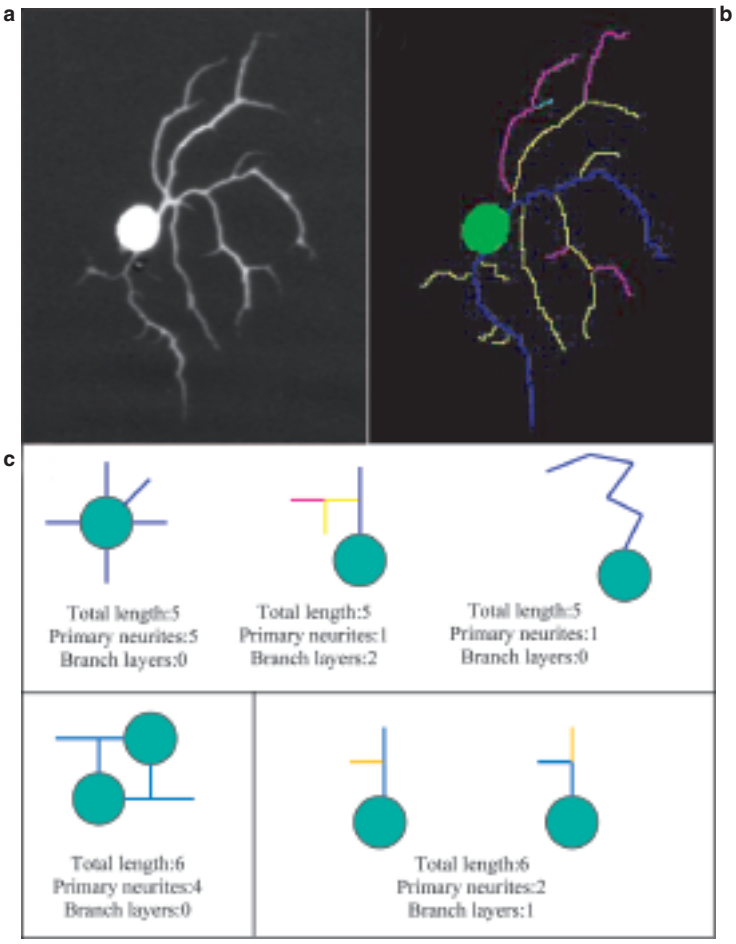

Figure 1 a) Representative image of a neuron viewed under fluorescence microscopy. b) Output generated by the software, detailing primary (blue), secondary (yellow), tertiary (purple), and quaternary neurites (teal). c) Examples of neurite trees, together with some features measured by the software, illustrating that the total neurite length is often inadequate to map significant differences in neurite structures.

Discriminating subtle changes

The most widespread feature describing neurite outgrowths is their total length. Occasionally, the number of branch points is also reported. With only these measures, cell morphologies that appear distinctly different to a human observer can end up sharing the same set of features, and hence be considered identical by an automated classifier downstream. There is thus a need for additional, biologically relevant features to characterize the branching structure of neurites.

The software reports the number of primary (or root) neurites as well as the number of layers associated with these primary neurites (secondary, tertiary, quaternary, etc., branches). These additional features allow users to selectively screen for compounds triggering different types of neurite outgrowth behavior, as shown in Figure 1. Results are available either on a cell-by-cell basis or as averages over images.

Improved robustness toward artefacts

The sophisticated measures in the software are more robust toward artefacts that commonly obscure the results of screening experiments. For instance, even at moderate cell plating densities, the neurites growing from one cell can intersect with those growing from a cell nearby. These intersections are traditionally mistaken for branch points. The software correctly differentiates between true neurite branch points and artefact branch points, making the measurements more robust toward variations in cell density than the simple branch point counts used in other systems. As a result, dose-response curves tend to have narrower error bars, and Z' factors (an assay quality statistic describing the separability of positive and negative controls) tend to be higher.

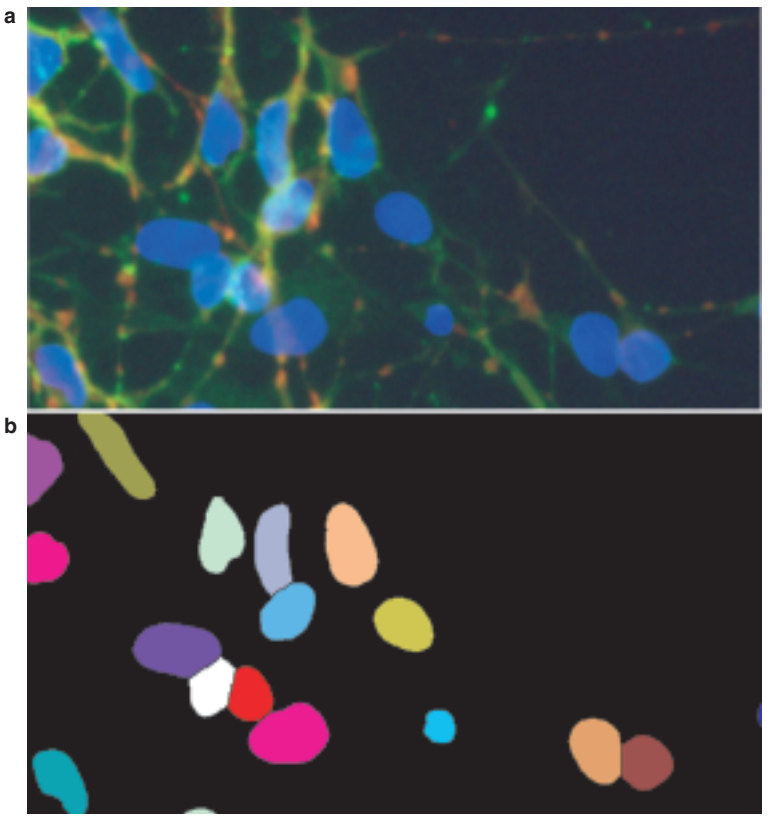

Figure 2 a) and b) Nuclei stained with a specific dye 4'-6-diamidino-2-phenylindole (DAPI). The software uses novel algorithms to exploit these nucleus channel images to segment cells reliably, resulting in accurate per-cell results.

Conclusion

Ideally, results should be available on a per-cell basis to correct for variations in cell plating density. A common problem here is the presence of closely touching neurons, which can be difficult to segment and result in biased per-cell measurements. The software's code for separating touching objects is based on a modified version of the watershed transform (*Figure 2*). The software can optionally accept nucleus channel images as input to make the cell segmentation process even more robust.

The authors are with the Biotech Imaging Group, CSIRO Mathematical and Information Sciences, Locked Bag 17, North Ryde NSW 1670, Australia; tel.: +61 02 9325 3208; fax: +61 02 9325 3200; e-mail: pascal.vallotton@csiro.au. *The software described here has been licensed into systems designed for secondary drug screening: ImageXpress™ (**Molecular Devices,** Sunnyvale, CA) and Pathway Bioimager™ (**BD Biosciences,** Franklin Lakes, NJ). It is also used in the Opera™ system (**Evotec Technologies,** Hamburg, Germany), which is capable of primary screening speeds. A standalone version of the software will be released for the American Society for Cell Biology meeting, San Francisco, CA, December 10–14, 2005. Images for this article were kindly provided by **Evotec Technologies**.*