HCA-Vision puts neuron images under a virtual scalpel

A new high-content-analysis program lets users formulate complex queries within the software in order to unlock information contained in images of cultured neuron cells.

by **Pascal VALLOTTON**, **Changming SUN**, and **Dadong WANG**

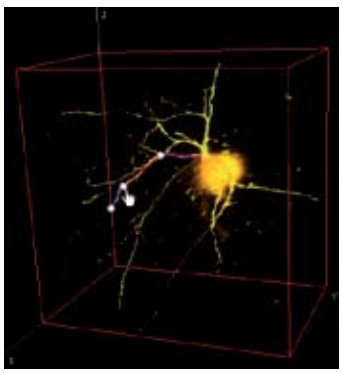


Image courtesy of Jenny Gunnersen, Howard Florey Institute, Melbourne, Australia.

Figure 1: Screenshot of HCA-Vision. Colours indicate different branching levels. For this cell, total neurite length was 3,455 pixels, maximum neurite length was 1,159 pixels, maximum branching level was 3, mean branching level was 1.45, number of branch points was 15, number of root neuritis was 6, number of neurite segments was 36, number of extremities was 19; the same information was available layer by layer but is not shown here. The maximum intensity along neurites was 254, the mean intensity was 64, and the integrated intensity was 262,232 grey levels.

The Division of Mathematical and Information Sciences (CMIS) at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia is hard at work to gain access to the vast amount of information contained in images of cultured neuron cells. The research seeks to aid in the discovery of new and better drugs, clarify mechanisms of action of existing drugs, and gain quantitative understanding of cell biology. This effort has resulted in an image-analysis package (Figure 1) dedicated to measuring various statistics describing neurons, including neurite lengths, number of branch points, and mean numbers of branching layers.

The increase in the ageing population of developed countries has resulted in growing incidences of such brain-related diseases as Alzheimer's, Parkinson's, and strokes. Preventive measures, better diagnostics, and effective treatment consume huge amounts of allocated resources. In this context, a considerable demand exists for tools that can quantify the action of various interventions on patient health.

Cell cultures of neurons play an important part in both drug development and the relatively new industry called high-content analysis (HCA). In HCA, robots manipulate cells, expose them to various candidate compounds, acquire images of these cells, and store those images in large databases for further analysis. Several hardware manufacturers offer commercial platforms that include integrated image-analysis capabilities, relying on algorithms developed at CMIS. The CSIRO division recently launched its own standalone software for high-content analysis called HCA-Vision, which can analyse images of cells any hardware platform generates.

The most conspicuous feature of neurons is their neurites, hair-like projections developing into complex, branched structures that mediate electrical and chemical signalling – ultimately, the very fabric of thought. Reports show the centrality of the dynamics of this intertwined network to cognitive processes, including learning. Compelling evidence shows that neurobiological dysfunctions link to defects in inter-neuronal communication. The relatively recent discovery that adult neurons can regenerate offers hope for patients affected by spinal cord injury. Determining neuritic behaviour will help researchers understand the mechanisms in neural damage and healing, with the long-term aim of developing therapy.

Neurites, being very thin structures, display weak and unclear contrast on highly variable backgrounds during imaging. Changming Sun at CMIS has recently developed a very fast algorithm capable of dealing with this complexity using non-maximum suppression as a mechanism to identify candidate pixels belonging to linear features. This is followed by several post-processing steps jointly developed and refined by the authors, aimed at completing and cleaning the neurite traces.

For example, Figure 3A illustrates how the non-maximum suppression algorithm has missed several linear features (white arrows). The HCA-Vision gap-closing algorithm based on a dynamic programming search of the optimal path can successfully close these remaining gaps (Figure 3B). Closing gaps is very important as, otherwise, whole neurite trees could be missed in the analysis and therefore bias the statistics.

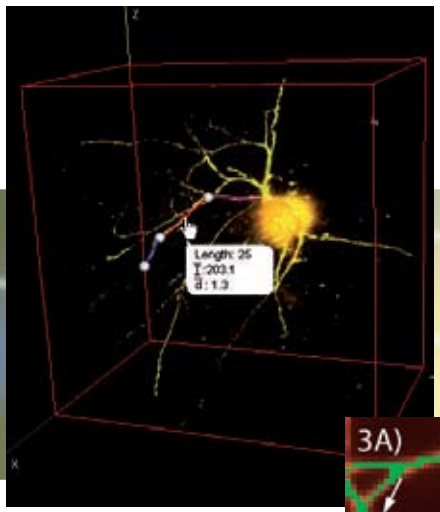
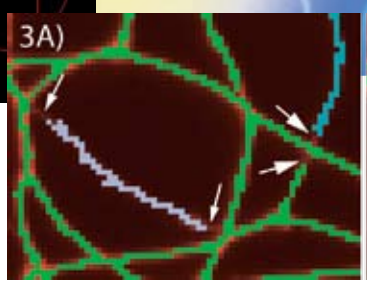


Figure 2: HCA-Vision allows measurement of neurons in 3D. The image shows a pyramidal neuron, filled with Alexa594 dye.



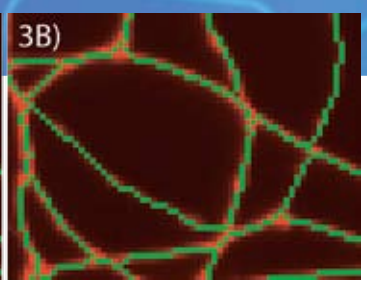


Figure 3: (A) Some linear features are missed by the non-maximum suppression algorithm (highlighted by white arrowheads). HCA-Vision's gap closing post-processing algorithm completes the missing stretches by extending existing traces optimally using dynamic programming. (B) The original image is shown in red, while segmentation results, as determined by the software, are overlaid in other colours.

Data courtesy of Pankaj Sah, QBI, Brisbane, Australia.

For most image-analysis algorithms, a number of parameters control the process and influence the quality of the results. The parameters of CMIS algorithms remain mostly independent of one another and can be adjusted successively in interactive help-utility wizards where users can pull on sliders to see, in real time, the outcome of selecting particular parameter values. This intuitive user-friendly system incorporated in HCA-Vision offers great improvement on the relatively intimidating parameter panels widespread in the industry.

The product targets neuroscience researchers interested in quantifying their images. It is particularly useful for members of the HCA community who demand high-quality image analysis and require tight control over the processes. HCA-Vision, developed under Microsoft .NET platform, is currently available for Microsoft Windows. It can read images in most formats, and its creators have made available an array of pre-processing tools to filter, resize, and transform images. The software offers full-batch-processing capabilities to analyse thousands of images in a very short time, a process supported by parameter profiles that users can store, communicate with others, and use for auditing purposes. They can export results in formats ranging from Excel tables and Crystal reports to various result images. Statistics that describe neurites at different levels of organisation are available on a cell-by-cell or population basis as is detailed information on cell bodies.

Eva Gak and her team at the Sheba Medical Center in Israel use HCA-Vision to characterise images of cultured neurons of marine mollusc *Aplysia* used as a model system to express genes associated with human neuropathologies. The product includes a utility to convert such images into pseudo-fluorescence contrast, thus permitting further processing. The CMIS researchers can distinguish subtle morphological differences between native *Aplysia* neurons and neurons expressing specific human genes. The team also compares HCA-Vision results with manually obtained results on data produced at the Howard Florey Institute in Melbourne by Jenny Gunnerson and co-workers. The larger scales of the analyses that they are now able to perform can, they hope, detect effects statistically insignificant under a smaller, manual-sampling regime. Figure 1 presents results obtained with HCA-Vision.

A sad but widely acknowledged fact is that software users have little interest in features available in existing HCA products. In fact, the immense diversity of biological questions they wish to address often forces them to build their own solutions. To avoid this trap, CMIS developers intend to implement HCA-Vision capabilities that will enable users to formulate any query within the software, however complex, supported by standard database technology. This scheme will also allow the development of a protein-translocation module in HCA-Vision that will broaden the applicability of the platform beyond neurite measurements.

The software has the potential to become a key tool for research in cell death, inflammatory response, cancer biology, and other biological areas. Interested parties can request a trial version of the software from the authors (hca-vision@csiro.au). The researchers are currently working on a 3D version of HCA-Vision expected for release early 2007 (Figure 2).

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