Image Analysis gives a clear view in research

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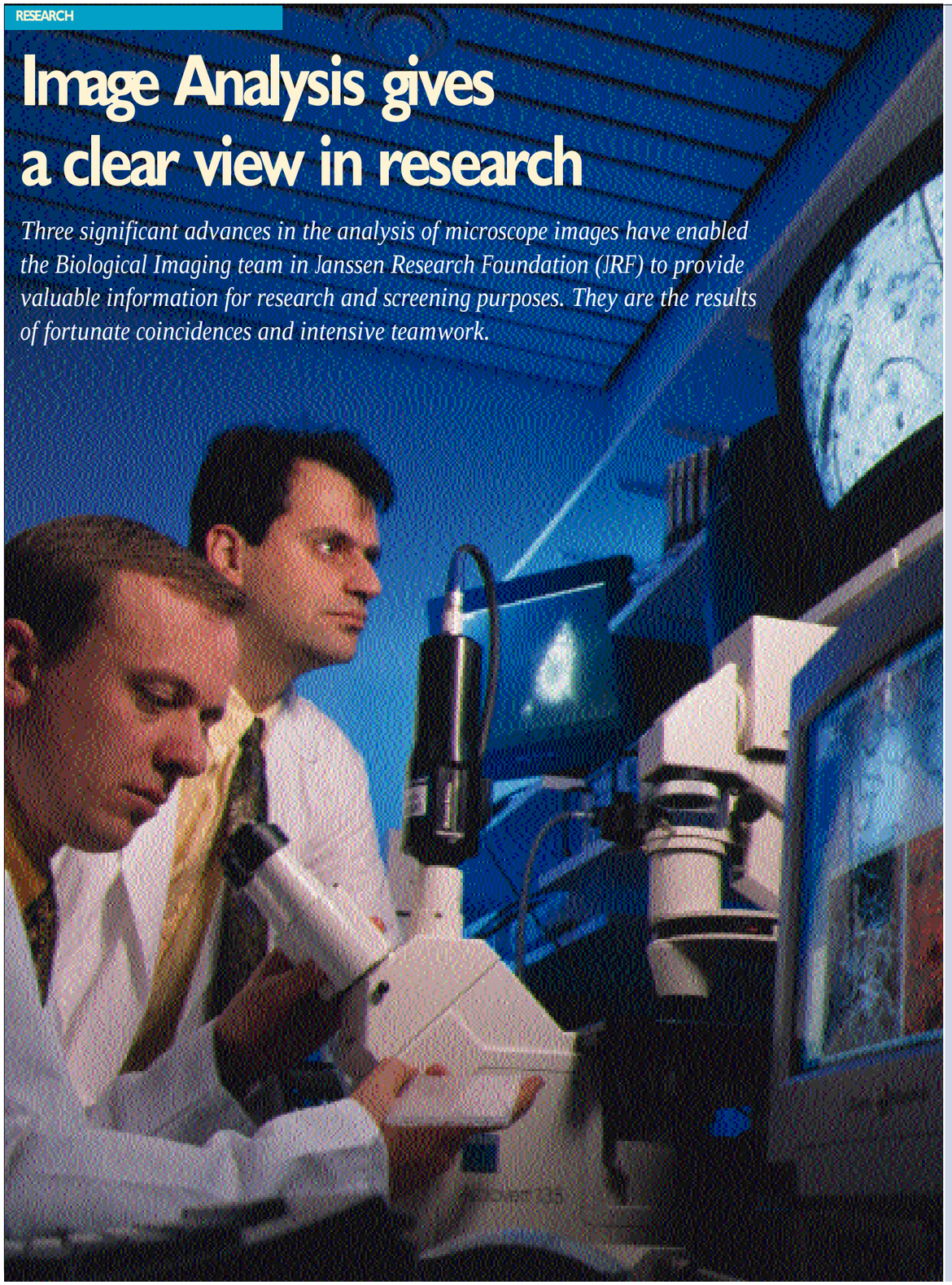

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Peter Van Osta, of the Biological Imaging Laboratory (BIL) in the Department of Life Sciences explains, "We have been able to build on a programme of continuing development in automated microscopy at JRF, which began some 15 years ago with Frans Cornelissen working for Marcel Borgers and Hugo Geerts. The current work is using these automated systems and is extracting quantitative results automatically through analysis of the images."

At the heart of the image acquisition system is a set of hardware comprising a standard Zeiss microscope, with a Maerzhauser motorised stage, a Silicon Graphics 02 workstation and a CCD camera.

"The image analysis software is based on a commercial program called SCIL Image but over the years JRF researchers have modified this extensively so that now there are more than 150 in-house image analysis routines, making the program truly unique," continues Peter Van Osta.

"The recent advances came about because of a fortunate coincidence that presented us with specific image analysis problems at the same time as we had a rare combination of talents present, including a PhD student, Jan-Mark Geusebroek, whose ability to develop effective fundamental algorithms made a crucial difference.

"The first key advance was a significant improvement in auto-focusing. Clearly, any automated microscopy must have automatic focusing but often the images we work with are, at best, very faint and blurred. In a project that required analysis of images of nematodes in the wells of multiwell assay plates we had an excellent example of a faint and blurry image. A robust algorithm developed by Jan-Mark led to auto-focusing that is both accurate and fast and will find the best focus in virtually any sample. Janssen has taken out a patent on this technology, which is a long way ahead of any commercially available software.

"The second key advance is referred to as multi-mode mosaic analysis, or M3 analysis. In automated microscopy, a system traditionally identifies objects in a single image, a cell or a tissue region. However, at high resolution the image to be viewed represents many times the field of view of a microscope. M3 analysis is a framework that enables the software to tile together adjacent images into one large image and to process several of these mosaic images in parallel. It is being used in tissue morphology and cellular assays, locating and identifying tissue regions and cells and measuring them, even distinguishing between multiple fluorescent labels. As a result, the development of an analysis that previously took several weeks is now finished in one week or even a few days by using this M3 framework.

"The third key advance is referred to as Scale Space or Spatial Modelling. This is a form of mathematical analysis of an image to produce a topographical representation where different objects are identified based on their geometric appearance, e.g. neurites as lines. The key benefit is that it overcomes the problems of changing conditions, such as fluctuations in levels of illumination.

"Using theoretical developments from Jan-Mark Geusebroek we have developed a system that will achieve a robust analysis even in faint and 'noisy' fluorescence microscopy images. It has been used, for example, in nuclear localisation screening where we track the movement of a labelled protein into and out of the cell nucleus.

"A further development on this theme has come with Colour Scale Space analysis, where the measurements are in terms of spectral colour. This concept is being used in color microscopy, either tissue morphology or fluorescence microscopy. The first application of this technique was in studies of cerebral blood flow in a project of Jos Van Reempts (Neuropathology).

"The results from these automated analyses can be entered automatically into statistical and spreadsheet programs and, as they are robust systems that will cope with changing conditions, they are ideal for screening, where reliability and speed are important," adds Peter Van Osta.

Kris Ver Donck (Senior Scientist in Advanced Biotechnologies), one of the customers of the BIL, describes some current applications of automated image analysis. "The nematode C. elegans is widely used in cytological studies as the entire nematode is just 1mm and can be viewed easily yet it has most major cell types as, for example, a mouse and it has a life cycle of just 3.5 days. This enables us to conduct in vivo studies at organ level, using fluorescent markers to reveal their size and shape for automated identification and measurement. We can also examine features such as the neuronal pathways, which have been fully mapped.

"Automated image analysis is being used to identify compounds that influence the synthesis or functioning of target proteins. For instance, a pair of muscles in the nematode normally forms an X shape. We studied a protein that, if it is defective, inhibited or absent, leads to an abnormal shape in these muscles. C. elegans nematodes were incubated in the wells of a micro titre plate, each well containing a different compound. We required all the key advances to automate the analysis of the results — auto-focusing, tiling and analysing the images. In fact it was this project in particular that stimulated the recent advances in auto-focusing.

"In a study on a messenger protein involved in inflammation pathways, NFkB-P65, we have developed an assay that reveals its movement into and out of the cell nucleus. The development of the Scale Space analysis made this much easier and enabled us to run large screening projects looking for inhibitors of the translocation. We screened 15,000 compounds in a fully automated project and identified 380 first time hits. These have been refined down to four top candidates, which are now completing profiling procedures.

"We have also used these automated image analysis techniques to help confirm in vitro results with protein P53. Normally P53 production is stimulated by damage to DNA and it acts as a tumour suppressor. The in vitro work was aimed at identifying compounds that would stimulate its production and we were able to demonstrate that the selected compounds were effective.

"Another application concerns cell population statistics. Here as many as 200 cells are held in each well and the technique is referred to as High Content Cell Screening (HCCS). It is not as fast as High Throughput screening but it gives far more detail, identifying sub-populations and presenting the data in a form that can easily be assimilated," says Kris Ver Donck.

Current developments will lead to improved image acquisition in High Content Cell Screening, tissue morphology, increased level of automation, and further progress with Scale Space and Colour Scale Space analyses. The Biological Imaging team is also working on automated analysis of 3D images from NMR studies, confocal imaging, sub-cellular fingerprinting and tracking proteins to specific cell locations other than the nucleus.