Fluorescence activated cell sorting facility.

Fluorescence microscopy floats free of limitations

No more than a nuisance to microscopists in the early 20th century, fluorescence has come of age in biology. It can now be manipulated and imaged with a precision that has thrust fluorescence microscopy to the forefront of life sciences researchs. The AUTOMATION consortium – with expertise in cell biology, microfluidic and di-electric cell handling, image processing, tomography and electron-multiplying charge-coupled-device (EMCCD) camera technologies – aims to liberate 3D fluorescence microscopy from its limitations.

Fluorochromes are dyes that fluoresce under illumination with ultraviolet or visible light of the right wavelength. By tagging the right fluorochromes to the right parts of a cell's anatomy, cell biologists can selectively reveal the specific details – and only those details – they need to observe; all else can be left dark. At the forefront of the methodologies, three-dimensional fluorescence microscopy' is now a commonplace laboratory tool which could not have been realised without advances in optics and microscopy techniques paralleling those in fluorochrome technologies. In its simplest form, 3D microscopy uses excitation illumination as normal to yield a fluorescent image, but the focal plane of the objective is mechanically moved in controlled steps whereby, instead of a single image, a series of images are acquired from each different focal plane through the z-axis of the sample. This 'through-stack' image series of so-called 'optical-sections' can then be reconstructed to map the 3D structure of the fluorescent signal.

Inherent flaws

Inherent flaws limit the performance of most 3D microscopes. For example, resolution along the z-axis – the axis along which the objective lens is moved to change focus – is always smaller than in the x-y plane. Other flaws are down to the object itself, as when optically dense parts get in the way of other parts further from the objective. For non-confocal systems, there are problems with out-of-focus light. Correction is possible, but only imperfectly, because the proper correction varies as a function of the position of the objective lens. Chromatic aberration, another flaw, can be corrected too, but again only for one position of the objective at a time. At the root of these problems, and least considered in alternative strategies, is the fact that all conventional methods for high-resolution 3D microscopy require that the sample, a living cell, must be immobilised during the acquisition, usually by adhesion to a glass cover slip or capillary. Already dependent on multiple correction techniques and assumptions, 3D imaging using through-stack procedures can ill afford the extra complexity of correcting for any motion of the object. For these reasons, non-adherent cells are out of bounds.

Move the object not the lens

Through heavy investment in component technologies, commercial systems design

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Coloured karyogram. © Institute for Medical Biology and Human Genetics, Graz

AUTOMATION
NEST ADVENTURE

AT A GLANCE

Official title
A novel imaging system providing high-content high-throughput multi-dimensional analysis of microscopic biological structure inside non-adherent living cells

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and a multi-disciplinary research effort, the AUTOMATION consortium expects to address these problems with a novel 3D fluorescence tomography approach. The design proposed is based on a configuration for which two of the consortium members have already secured a European patent. It differs from conventional systems in one fundamental respect. Once the first section of the sample has been captured, subsequent sections for any 3D image are obtained by moving the object – not the objective lens. Thus, the object's position is not fixed, but instead controlled by di-electric fields generated inside a micro-electrode cage inside a microfluidic chamber. Under development by German partner, Evotec Technologies, this cell-manipulation technology will eliminate the problems associated with refocusing movement of the objective lens between sections, and open the way to single-cell tomographic imaging.

The project's aim is to introduce a new 3D imaging methodology using isotropic-optical sampling and mathematical reconstruction, enabling true single-cell fluorescent tomography.

Assays for non-adherent cells

Single-cell 3D tomography is envisaged to work similarly to 3D image tomography, for example, whereby a tumour inside a patient can be visualised by computing images recorded using an X-ray scanner that rotates around the body. The partners already know that di-electric field control gives predictable control, allowing micro-translation and micro-rotation of single cells, and their ultimate aim is to construct, from the sections thus obtained, a 3D map of fluorescence from the sample under the lens. To achieve this, AUTOMATION will do new research to provide a mathematical basis for tomographic reconstruction of single-cell fluorescence, visualised using micro-rotation. Their success is likely to lead to applications in biomedicine and cell biology. Notably, by automating micro-rotation imaging and reconstruction, this system will provide a new cell-based assay tool, capable of accelerating clinical diagnostic analysis of blood and biopsy samples. Equally, for pre-clinical screening assays using non-adherent cell models and fluorescent tags, the device will enhance basic research on cell structure and function. Target cells include stem cells, embryos, lymphocytes and other blood cells – all of them important in cell-based assays and diagnostics, and all non-adherent. The long-term impact of AUTOMATION is most likely to come in the fields of diagnostics, drug discovery, therapeutics and basic research.

SIXTH FRAMEWORK PROGRAMME