High-content analysis with AxioVision ASSAYbuilder™

In drug discovery, and in biological experimentation in general, cell-based assays are replacing conventional *in vitro* binding or enzyme assays more and more. New fluorescent probes as well as recently developed fluorescence imaging techniques are the basis for image-based high-content analysis (HCA). But automated extraction of biologically meaningful data from images still represents one of the most important challenges for researchers. AxioVision ASSAYbuilder™ is a new software module that fills this gap and efficiently produces high-content information on drug responses from cell-image data.

HCA is a combination of cell-based assays, (high-resolution) fluorescence imaging, automation and advanced image analysis¹. It has been widely adopted in the pharmaceutical industries for target identification and validation. Furthermore, in secondary screens HCA can be used to detect potential toxicities or identify a compound's mechanism of action. HCA can be used to measure multiple biological pathways simultaneously or to reveal off-target drug effects².

There is a growing need to combine research imaging with high-content analysis or screening on the same equipment—for example, to reduce necessary investments or to keep learning curves low. Dual-use imaging systems thereby dramatically increase productivity and efficiency. A solution that addresses this need consists of the Carl Zeiss Cell Observer[®] and the new AxioVision ASSAYbuilder HCA module.

ASSAYbuilder features

Meaningful biological data can only be extracted from high-quality images, stressing the need for cutting-edge imaging systems.

The Carl Zeiss Cell Observer was developed for observing and documenting living or fixed cells, organisms and intracellular processes in several dimensions. The Cell Observer enables fully automated acquisition of up to 32 fluorescence or bright-field channels, z-stacks, time-lapse images and any number of positions of a specimen—for example, from pre-stored plate formats. A variety of fluorescence illumination sources, including the new Colibri LED light source and optional components such as the ApoTome optical sectioning device or incubators, further expand the possibilities for experiments with Cell Observer.

AxioVision ASSAYbuilder (powered by Cellomics[®]) is a workflow-oriented software tool that makes sophisticated object-based image analysis possible for a wide range of high-content screening assays. The module provides intuitive, preprogrammed, workflow-oriented, object-based quantitative analyses of multichannel fluorescence and bright-field images

Figure 1 | ASSAYbuilder application layout screenshot. Above, untreated cells in the control image (left) together with corresponding histogram data (right). Below, a stimulated sample with cells that exhibit a relative increase in nuclear fluorescence. Nucleocytoplasmic translocations such as are observed here typically occur in the process of activation of many transcription factors.

with cross-channel referencing and hierarchical data output (Fig. 1).

Applied either to motorized systems, such as the Cell Observer, or to manual systems with a digital imaging camera, ASSAYbuilder imparts the capability to run measurement protocols on a large number of individual experiments. The images are acquired using AxioVision advanced image acquisition functions or can also be imported from Carl Zeiss confocal microscopes.

Depending on the desired application, images are analyzed with one or more of five different ASSAYbuilder Analyst modules: Physiology Analyst, Morphology Analyst, Membrane Analyst, Cell Cycle Analyst and Motility Analyst. The Analyst modules organize object-based output

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and analytical processes around common biological assays. Images and relational data are interactively linked and automatically presented, allowing rapid quality control. Also, user-defined demographic tools enable the researcher to collect high-content measurements of subpopulations.

Flexibility of application

Typical examples of applications of the AxioVision ASSAYbuilder module and the Analyst modules include analysis of apoptosis and cytotoxicity; cytoskeletal arrangement and molecular patterning; DNA damage; receptor activation and intracellular signalling; biosensor expression or localization; neuronal processes; cell colony and organism formation, and angiogenesis; colocalization of targets and target sequestering; cell cycle; tumor suppressors and oncogene expression; and cell motility and wound healing.

ASSAYbuilder performance

ASSAYbuilder can, for example, be used in cell-cycle analysis and to monitor G protein-coupled receptor (GPCR) internalization—two cell-based applications relevant for drug discovery.

Cell-proliferation and cell-cycle analysis is one major focus of drug-discovery research, as uncontrolled proliferation is a hallmark of cancer cells. HCA can be used to determine cell-cycle state and to quantify cell proliferation activity by simultaneously measuring DNA content and correlating multiple parameters relating to the cell cycle.

We have used hepatocytes to determine effects of ‘medicinal’ plant extracts on cell-cycle state. We plated cells in 96-well cell-culture plates and treated them for 24 h with the extracts. Subsequently we stained the cells and imaged them using a Cell Observer with a scanning stage. HCA with ASSAYbuilder Cell Cycle Analyst revealed effects on cell proliferation and cell cycle (**Fig. 2**).

Another major focus of drug discovery research is finding more effective drugs that act on GPCRs; approximately three-quarters of today's drugs act on these targets. ASSAYbuilder Physiology Analyst is able to detect and classify different subcellular distributions of receptor signals (for example, endosomal localization) and thereby provides a fast and robust analysis of GPCR activation (**Fig. 3**).

Conclusion

AxioVision ASSAYbuilder is a workflow-oriented HCA software module that does not require specialized knowledge in image analysis, scripting or programming. It guides the user through the process of assay development. All important steps in high-content image analysis are predetermined and can then be optimized interactively by the user. As soon as the user is satisfied with the data generated from a limited number of exemplary images (for example, positive and negative control), the protocol can be saved and run over a multitude of folders with images.

Cell Observer, AxioVision and the ASSAYbuilder HCA module can be successfully applied for identification and characterization of potential drugs in cell-based assays. Notably, with the same equipment, highly specialized imaging tasks can be conducted to understand the pharmacological and biochemical mode of action of compounds—truly representing a dual-use research and screening system.

Additional information

Additional information on the Carl Zeiss Cell Observer, AxioVision and the ASSAYbuilder HCA module is available on the website <http://www.zeiss.de/high-content-analysis>. Cellomics[®] is a registered trademark of Thermo Fisher Scientific Inc. and its subsidiaries.

ACKNOWLEDGMENTS

I thank B. Kraus for providing samples, image data and comments on the text.

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