High Content Analysis with ASSAYbuilder™

A Solution That Will Take Your Research to New Heights.

The increasingly complex tasks faced in cell-based research demand increasingly sophisticated techniques in order to achieve meaningful, scientific results. High Content Analysis (HCA) with ASSAYbuilder™ from Carl Zeiss offers the most reliable way to obtain biologically relevant data from your image.

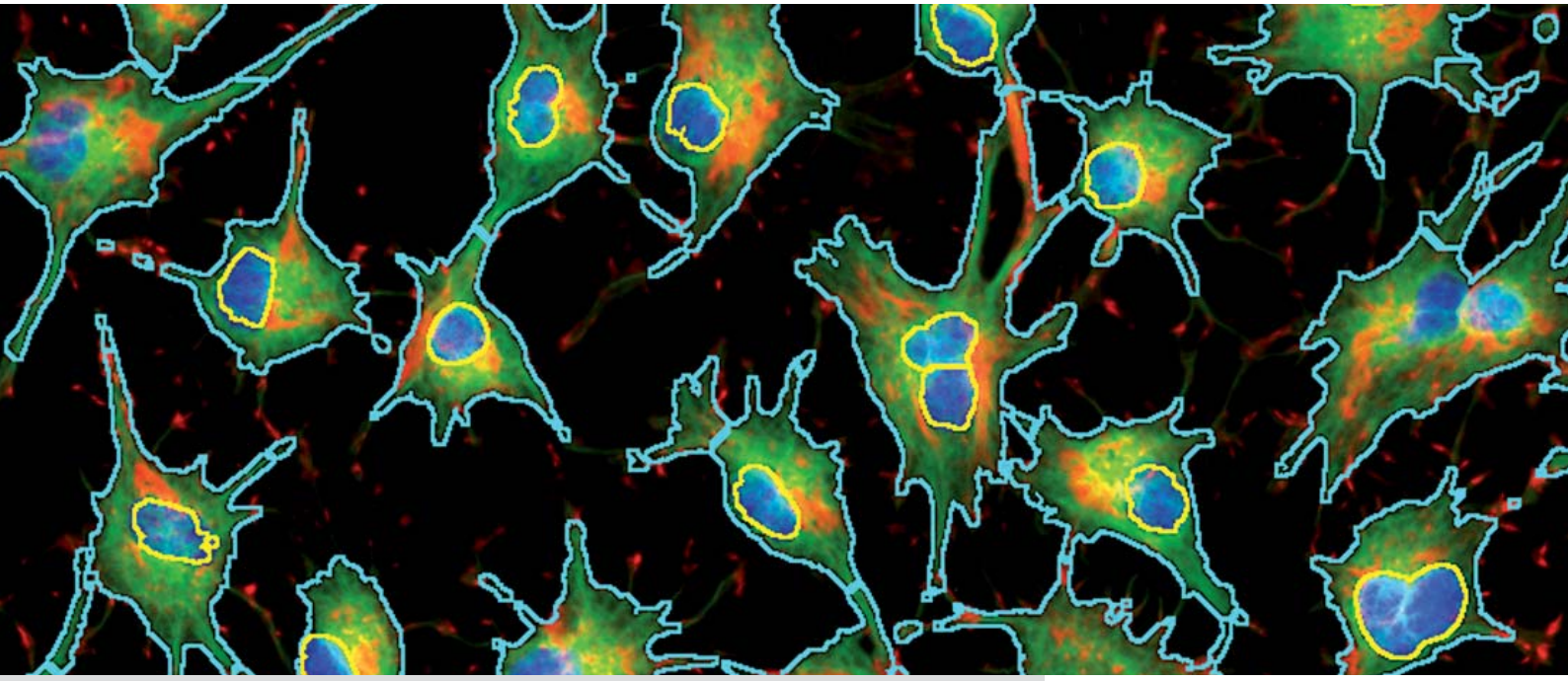
More information: from image to data with HCA

When answering highly complex questions, a first-class image is only the first step towards scientifically relevant results. After all, an image contains a wealth of hidden information which can only be revealed with considerable effort. The solution is High Content Analysis (HCA) with the specially developed ASSAYbuilder™ software module, in which cell-based tests (assays) are combined with high-resolution fluorescence images, automated workflows and complex image analysis. The result is that even unexpected, previously undetected phenomena can be uncovered.

Increased performance for biology

Developed together with Cellomics®, the pioneer of High Content Analysis, ASSAYbuilder™ combines 15 years' experience in HCA with many years' expertise in the development of high-performance microscope systems. The result is a powerful software module that is tailored in every respect to the very highest demands in the field of cell biology research.

- Fast and direct extraction of data
- Compatibility with many components and microscopes
- Intuitive operation with predefined workflows
- Functional packages for different applications



The Software Solution for Cell-Based High Content Analysis

ZEISS

We make it visible.

Versatile. Modular. Powerful.

Flexible in Research and Routine.

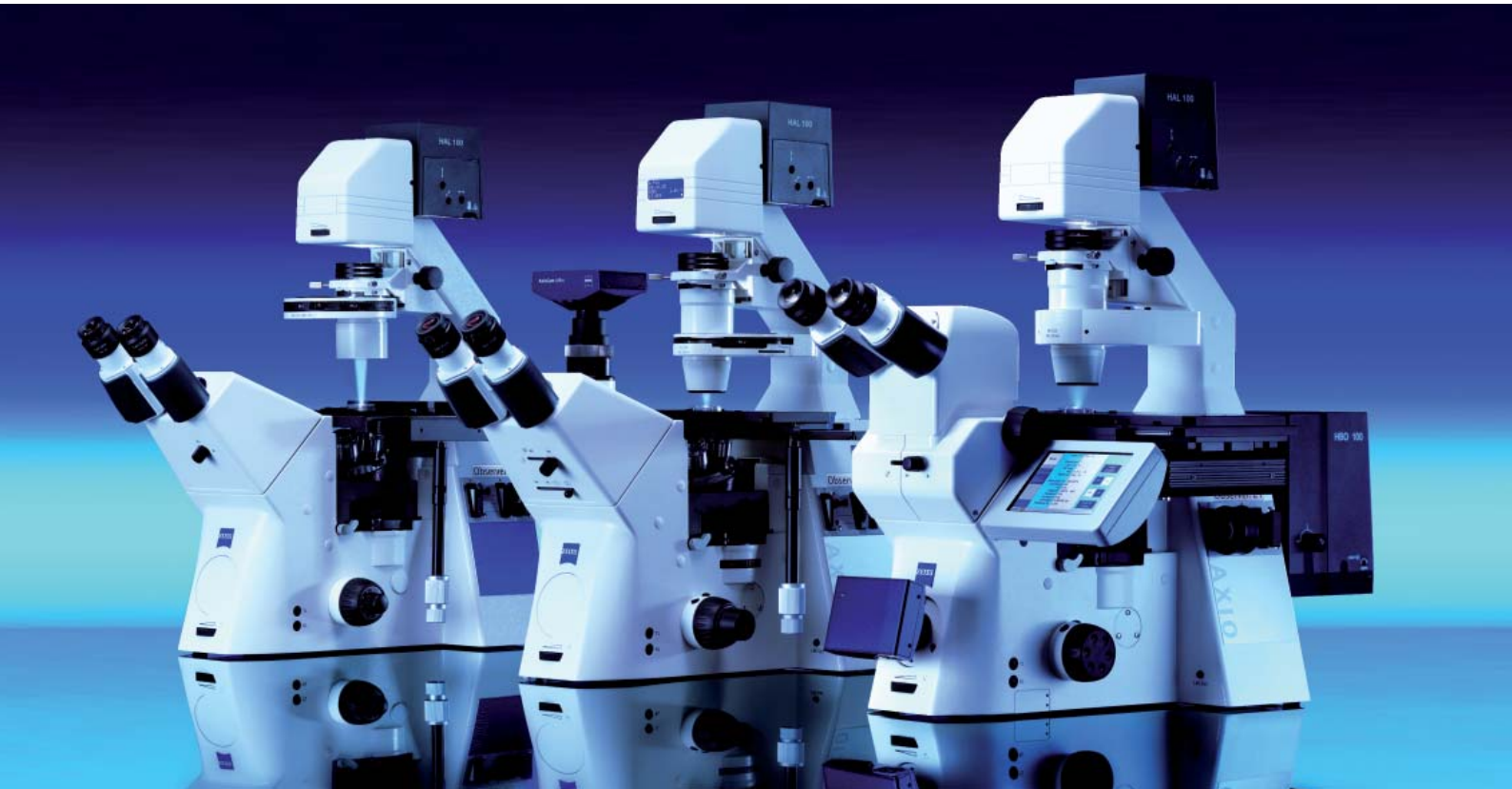
From research microscope to screening workstation: ASSAYbuilder™ can be integrated into existing Carl Zeiss microscopes and systems with no problem at all.

Analytical precision in all applications

With a range of key parameters describing an image, ASSAYbuilder™ delivers precisely the image data that you require as a basis for making decisions in order to plan further experiments or to give your publications scientific substantiation. Both image and data are available, in parallel, on your monitor throughout the entire analysis process, and that applies for a whole host of possible applications and experiments. With ASSAYbuilder™ you can analyze DNA damage, receptor activation, protein-kinase activation or transcription factor activation, molecular localization and translocation, colony formation, cell differentiation, reporter expression and much more.

Modularity equals economy and flexibility

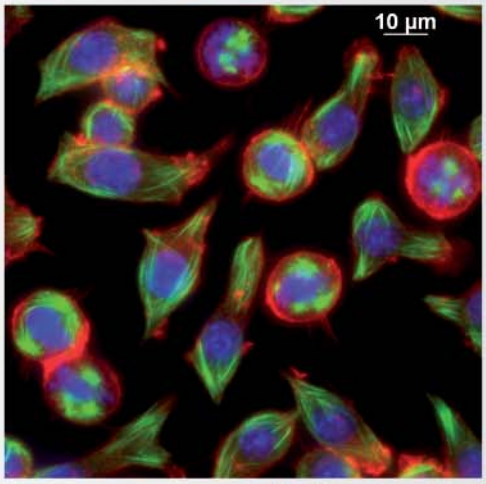
The modular architecture of the Carl Zeiss system opens up all the possibilities of the very latest research. Newcomers to HCA can combine ASSAYbuilder™ and a digital microscope camera with a routine microscope and perform initial analyses using a small number of samples. You can then expand your HCA system – module by module – as your applications call for it. For high-end research, Carl Zeiss offers individually configured High Content platforms, in every combination imaginable. These platforms offer enormous flexibility in terms of upgrading and retrofitting.

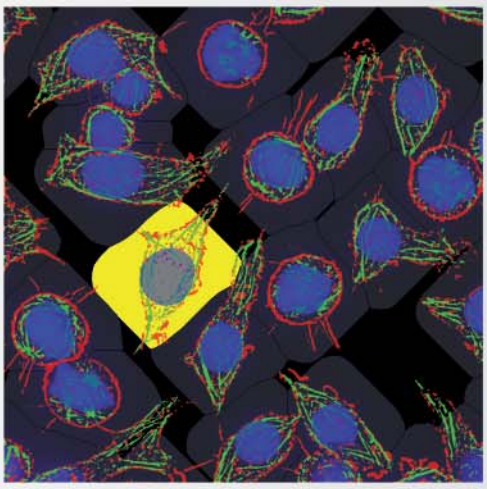


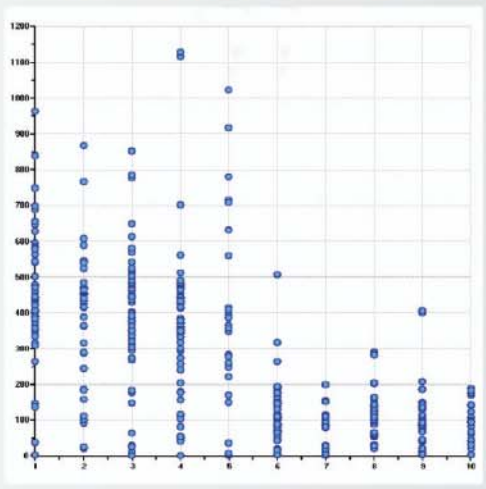
The direct way to obtain High Content Data from your sample

Thanks to its intuitive operation, ASSAYbuilder™ makes the procedure from specimen to analysis easier than ever before. Firstly, you acquire your images as usual. Then, depending on the task at hand, you select the correct analysis protocol, which can be modified if necessary. Should it not be suitable, generating a new protocol is a quick and simple process. You can check at a glance whether the type and quality of the data meet your requirements. To do this, use either a typical image or a small selection, e.g. two images of a sample with different phenotypes. If the quality is right, start the analysis of your images using the protocol. The data are directly transferred to and stored on your hard disk for display and further processing.

With brilliant image material, high-performance research systems and components provide the basis for scientifically precise analyses.







Selection of interesting region of sample

Image acquisition

Automatic image processing

Analysis protocol: development/ optimization

Automatic analysis of archived images

Display and export of data

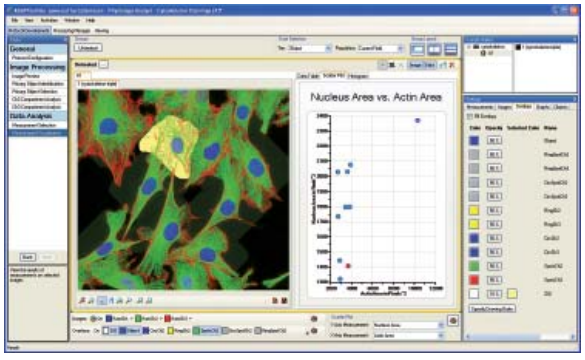
Special Tasks Demand Special Solutions.

Or One That Can Answer Every Question.

The diversity of the tasks undertaken in the field of life sciences is matched by the diversity of the applications offered by ASSAYbuilder™. The Analyst packages, which have each been tailored to address specific biological questions, cover your applications in the best possible way.

Analyzing with ASSAYbuilder™

Functional right down to the last detail – ASSAYbuilder™ meets the high demands that modern research tasks entail in terms of convenience, ease of operation and scope of performance.

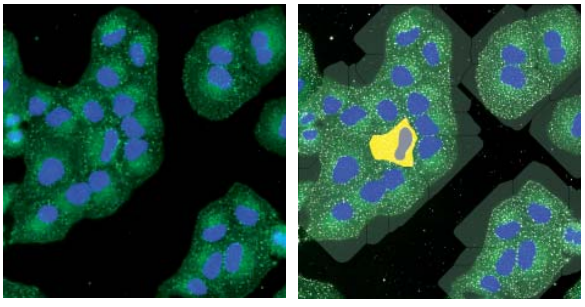


With ASSAYbuilder™ everything is in exactly the right place. Images are displayed in the central area of the user interface together with associated data. Around the outside you will find all the important setting options.

- Clearly structured user interface
- Intuitive guidance through the course of the experiment
- Image-based analysis taking the following levels into consideration: cell, image, well, timepoint
- Permanent linking of images and data
- Hierarchically organized data with several display and export options
- Five Analyst packages tailored to various tasks, each with specifically selected measurement parameters and procedures

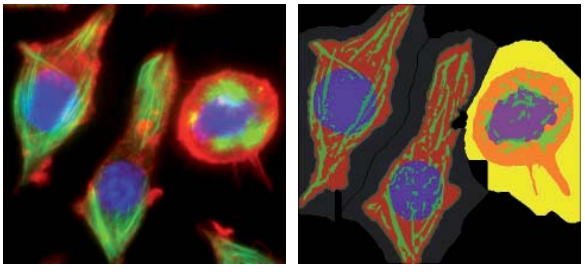
Physiology Analyst: the all-rounder

The most versatile of the ASSAYbuilder™ Analysts, the Physiology Analyst makes it particularly easy to enter the world of HCA. Using this package you can develop your own multiparameter analyses for quantifying the intensities of macromolecules in cellular compartments or the transport of macromolecules between compartments. The cells to be analyzed are labeled using a stain in one image channel. The Physiology Analyst then automatically recognizes and characterizes the cell population in a maximum of six channels. In this way, intensities, the number of spots, and many other parameters are determined for cells and cellular regions quickly and without the need for time-consuming workflows.



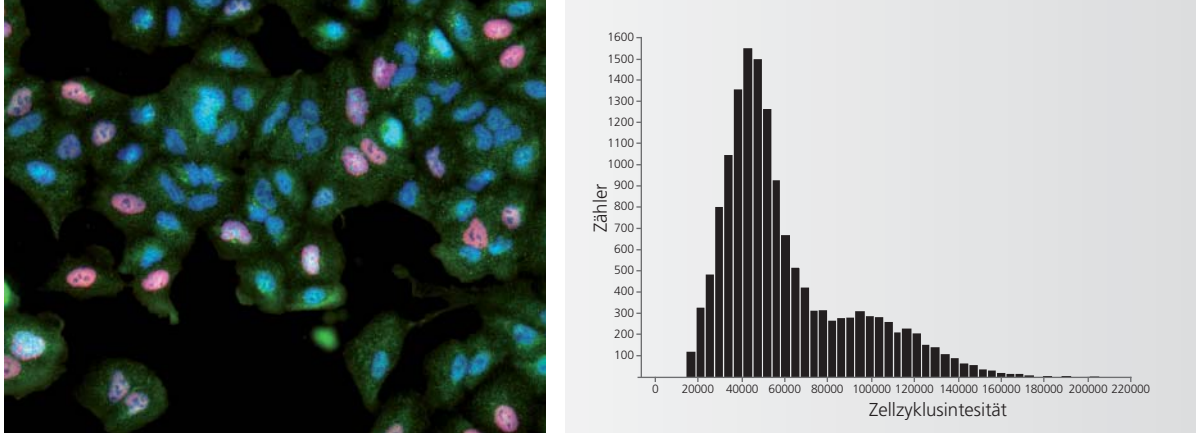
Fluorescence-stained cells before (left) and after object recognition by the Physiology Analyst. Cells: CHO with stained cell nucleus (blue) and receptor (green).

Morphology Analyst: the powerhouse



Fluorescence-stained cells before (left) and after object recognition by the Morphology Analyst. Cells: N11 microglia with stained cell nucleus (blue), microtubules (green) and actin (red).
Dr. Birgit Kraus, University of Regensburg, Germany

This Analyst is essential for complex analysis tasks as it covers an impressively wide range of different morphological questions. It enables the analysis of intracellular localization, as well as of the orientation and structure of cellular components such as organelles, macromolecular clusters or the cytoskeleton. The scope of this package also includes the analysis of the morphology of entire



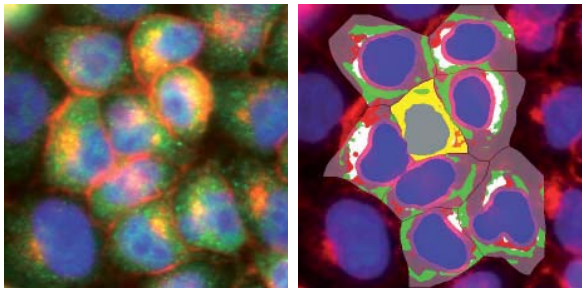
Left: image of a population of cells with stained nuclei (blue) and also exhibiting red BrdU fluorescence in certain cell nuclei. Cell Cycle Analyst calculates the DNA quantity from the intensity in the cell nucleus and presents this, for example, in the form of a histogram (right) for the entire cell population. This serves as a basis for calculations relating to the cell cycle status, which can be correlated with additional factors (e.g. with BrdU stains).

cells, e.g. describing form, texture and area, or of small organisms such as protozoa or C. elegans. Further analytical functions include intercellular distance, the characteristics of cell colonies, such as form, size and cell positions, and the arrangement of like and unlike cells within a mixed population.

Cell Cycle Analyst: the specialist

Cell Cycle Analyst can be used on its own or, for example, to supplement the data gathered from the Physiology Analyst. In detail, this package offers a multiparameter solution for analyzing the cell cycle of populations of adherent cells and which phase of the cell cycle individual cells are in. Following staining with an appropriate dye, this Analyst determines the DNA content and the fluorescence intensities of up to three cell cycle-relevant factors (labeled, for example, with immunofluorescence) in the same cell. It correlates the identified cell cycle phase of a cell with the presence or the activation status of additional cellular factors.

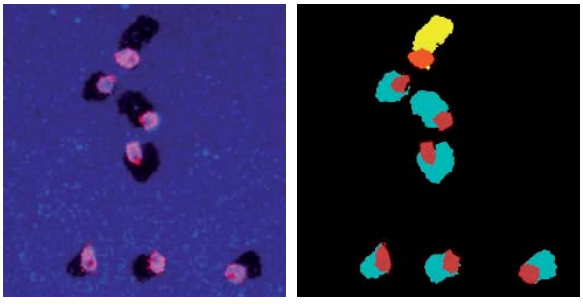
Membrane Analyst: the separatist



Fluorescence-stained cells before (left) and after object recognition by the Membrane Analyst. Cells: HeLa with stained cell nucleus (blue), protein kinase C (green) and membrane (red).

The Membrane Analyst is the perfect module for fast, complex colocalization analysis at single cell level. It analyzes signal translocations in the cell, particularly from cytoplasm to cell membrane and vice versa. To do this, you first define cells with a nuclear stain in one channel. You then define a second cellular compartment, e.g. the cell membrane, in another channel by means of a specific reference fluorescence. In a third channel, you determine the intensity of the macromolecules of interest in different cell compartments, such as the cell nucleus, the membrane or in cytoplasmic regions. Membrane Analyst establishes the signal intensities in the cellular regions and automatically calculates the redistribution of signals within the various cell compartments.

Motility Analyst: the pathfinder



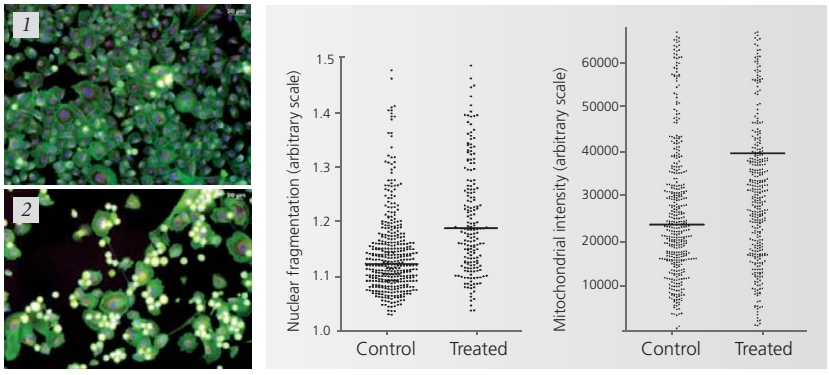
Fluorescence-stained cells before (left) and after object recognition by the Motility Analyst. Staining: lawn of blue fluorescent beads. Cells: N11 microglia with actin staining (red).
Dr. Birgit Kraus, University of Regensburg, Germany

The Motility Analyst allows you to determine cell motility or phagocytotic activity even without using the time lapse imaging function and without incubation on the microscope. Thanks to a special experimental process, cell motility and fundamental parameters of cell morphology can be analyzed. Living cells are plated on a lawn of fluorescent beads. The cells are then cultivated in their usual environment. As the cells move across the lawn, they leave behind a fluorescence-free track. After a predefined period of time, the cells are fixed and stained. Motility Analyst analyzes and measures the track left behind by the cells and quantifies their movement.

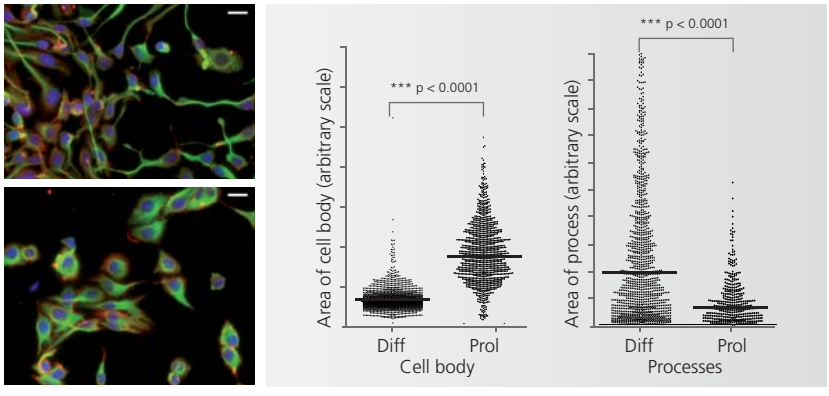
Analysis from A to Z.

A Complete Workflow Ensures Maximum Convenience.

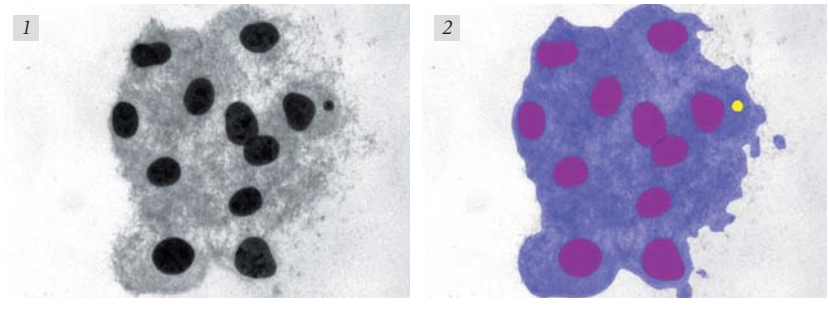
Your requirements determine which module you use – depending on the target parameters you require, you choose the appropriate ASSAYbuilder™ Analyst(s) for the task. All the relevant parameters are made available in a complete, universal and guided workflow – however complex your application.



Left: fluorescence-stained cells. 1) untreated control and 2) cells treated with a substance that triggers apoptosis. Cells: PC3 with stained cell nucleus (blue), actin (green) and mitochondria (red). The graph on the left shows the strength of the cell nucleus fragmentation as a measure of apoptosis, while the graph on the right shows the intensity of the mitochondria. Eva Gebefuegi, Helmholtz Center, Munich, Germany



Left: neural stem cells. As a result of cellular differentiation the morphology of the cells also changes. Proliferating cells have a large cell body but only slightly pronounced processes, while differentiated cells display the reverse characteristics. Ina Rothenaigner, Helmholtz Center, Munich, Germany (Publication: Rothenaigner et al, AIDS 2007, 21: 2271-2281)



1) Brightfield image 2) Nucleus and micronucleus identification (yellow) by Morphology Analyst

Apoptosis

Apoptosis, as a form of programmed cell death, is extremely important for the health of an organism. When a cell undergoes apoptosis, a large proportion of its properties change. Analyzing these changes can make a crucial contribution towards revealing the stage, extent and precise mechanism of the process. High Content Analysis with ASSAYbuilder™ offers a whole range of such parameters describing apoptosis and, consequently, makes both an early and precise analysis possible.

Cell morphology

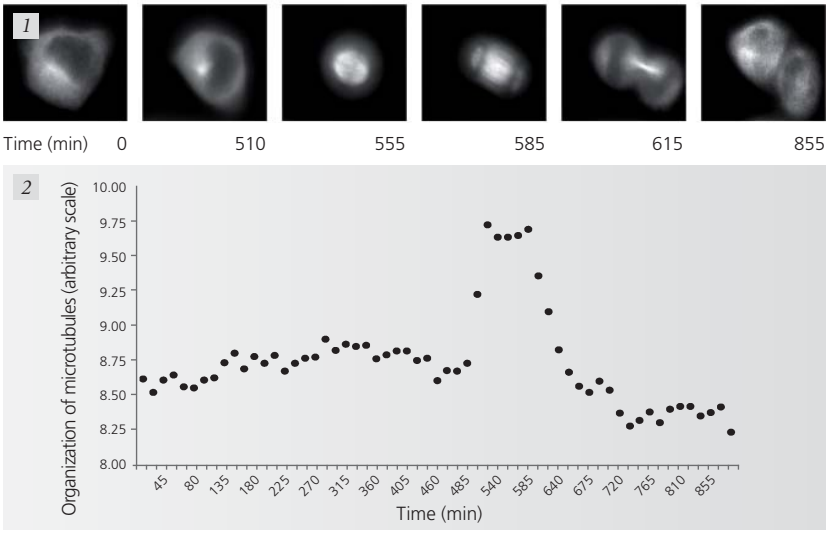
High Content Analysis also fills a gap in this area of research. By no means can all characteristics and changes in cell morphology be detected and determined with the naked eye. ASSAYbuilder™ analyzes biological objects objectively and precisely and delivers quantitative data for simple or highly complex measurements, providing a reliable basis for reproducible results and publications.

Micronucleus test

The micronucleus test is an established technique for testing mutagenicity. Using ASSAYbuilder™, the proportion of cells in a population can be determined in which an additional, abnormally small cell nucleus has developed during cell division.

Morphology of the cytoskeleton

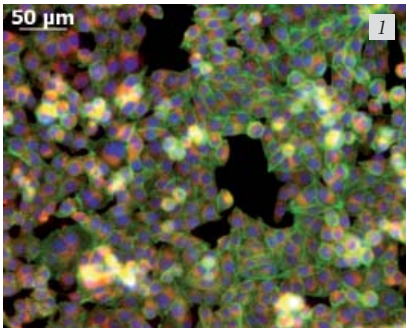
ASSAYbuilder™ also opens up new opportunities for cell and tumor biology, because it allows such complex parameters as, for example, tubulin polymerization or the organization of actin fibers to be described. The various changes to the cytoskeleton are consequently made accessible for research.

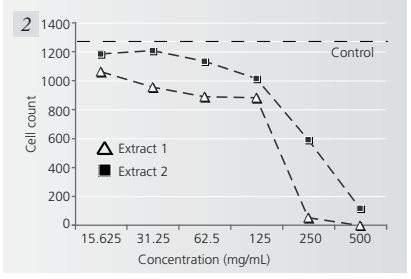


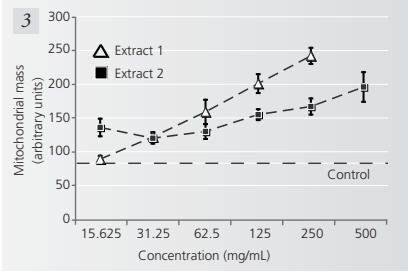
1) Frames from a time lapse image during the cell division of a HeLa cell with alpha-tubulin live cell stain. 2) Analysis of the organization of microtubules over time.
Dr. Birgit Kraus, University of Regensburg, Germany

Cytotoxicity

There are a number of tests for determining a substance's cytotoxicity; that is to say, it's ability to damage cells or tissues. However, no other method provides quite so much crucial information at once as High Content Analysis. Depending on the type of assay chosen, it is possible to determine the cell count, cell density, membrane damage, DNA damage, mitochondrial damage and much more – simultaneously. High Content Analysis with ASSAYbuilder™ provides you with the parameters to reveal the mechanism of cytotoxicity.



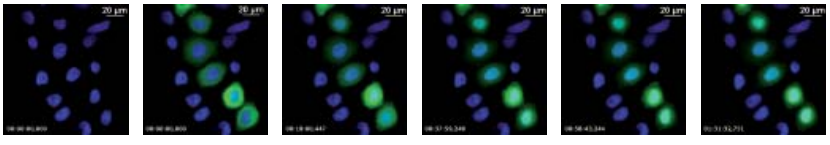


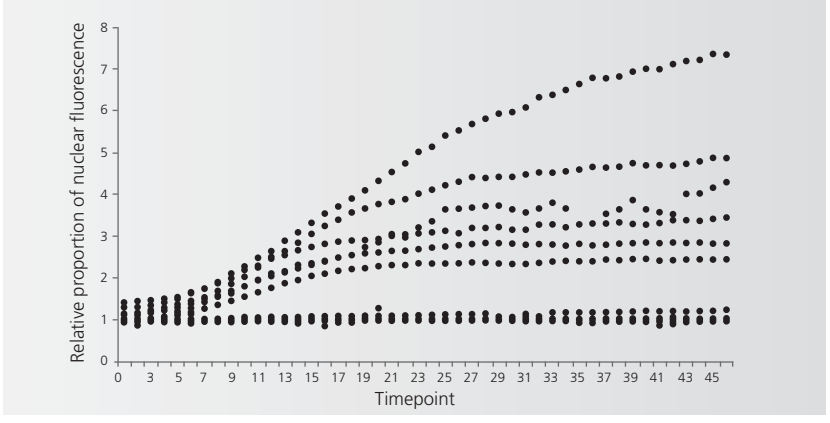


1) Huh-7 liver cells with stained cell nucleus (blue), microtubules (red) and actin (green).
2) When treated with extracts from medical plants, the number of remaining cells drops as the extract concentration increases.
3) The mitochondrial mass, an early symptom of cell-damaging processes, increases with increasing extract concentration. Dr. Birgit Kraus, University of Regensburg, Germany

Nucleocytoplasmic translocation

Translocations of factors within the cell take place during many cellular processes – whether from the cell membrane or nucleus into the cytosol and vice versa, or between different organelles and compartments. ASSAYbuilder™ analyzes endpoint assays or time lapse images of these processes and is also able to provide information on other cellular parameters at the same time, which help you to evaluate and classify the results of your experiment.





Frames from a time lapse image of HeLa cells which express a GFP fusion protein (green) and the nuclei of which have been stained (blue). After the addition of a substance, the fusion protein moves from the cytoplasm to the cell nucleus. The relative increase of green nuclear fluorescence can be analyzed over time and displayed in a graph for each cell (bottom figure).

Simply Perfect.

High Performance Through Selected Components.

In order to deliver a high quality result with seamless integration, each component of a microscope system must meet the highest requirements individually. The greatest possible flexibility in terms of combinations means the greatest possible range of applications. Carl Zeiss also offers you an impressive product spectrum for High Content Analysis.

Cell Observer®: new dimensions with HCA

Cell Observer®, based on the motorized research microscope Axio Observer, is ideally suited for HCA – as well as offering outstanding optical performance with excellent image quality and fast motorization, it is also ergonomic and extremely stable. A wide range of motorized stages are available for increased sample throughput and larger sample areas. For research under physiological conditions Cell Observer® offers a comprehensive incubation concept – from simple heating and cooling through to the detailed control of all key environmental parameters. Well thought-out accessories complete the modular concept: fast shutters and filter wheels, HBO and LED light sources, and many more components besides, offer you a system that you can expand with precisely those components that are required for your application.

Axio Imager: the upright research platform

Axio Imager also provides an ideal basis for HCA. Integrated into a system like Cell Observer® or as a high-end microscope, Axio Imager is particularly useful for applications which require high resolution images of fixed specimens on slides at high magnifications. Images can also be acquired from large slide areas using immersion

Cell Observer® with Incubator XL.
You will find more information on the comprehensive range of configurations in our brochure "Cell Observer® - Living Cells".

The LSM 710, the new reference in confocal microscopy.

You can use objectives with a very high working distance (left) or for multi-immersion (right) in order to acquire the best possible images for analysis.

objectives. If several types of specimen are being used, the motorized 6x or 10x reflector turret ensures maximum flexibility in terms of the dye combinations that are possible.

LSM: optimal image quality for difficult samples
Images acquired using confocal laser microscopy are unsurpassed in terms of resolution and quality, and you don't have to do without this just because you are performing HCA. The LSM series from Carl Zeiss can be easily combined with ASSAYbuilder™ in order to analyze thick tissue sections or to optimize image quality with spectral unmixing, for example.

SteREO: problem-free analysis of large specimens
The same applies to images acquired using stereomicroscopes, a technique that is necessary, for example, in the case of large specimens, long working distances, wide fields of view or low magnifications. In combination with AxioVision, you will acquire excellent image material for High Content Analysis.

Perfectly geared to HCA: the components
Within the Carl Zeiss product spectrum there is a whole range of components offering perfect support for your High Content Analysis.

- **Best optics**
  Carl Zeiss objectives are rated highly in the fields of science and research thanks to their excellent optical performance. They offer the highest possible resolution with the strongest contrast – in every application.

- **ApoTome**
  The standard for brilliant images in 3D fluorescence imaging delivers optical sections of your specimens, stray light-free and with brilliant contrast.

- **New light for fluorescence**
  Colibri, the newest light source from Carl Zeiss, makes use of high-performance LEDs rather than white light. An extremely stable light intensity, homogeneous illumination and images rich in contrast and with a high dynamic range create the optimal conditions for HCA.

- **Stable focus**
  Perfect freedom from drift in z: Definite Focus, an LED-supported focus system, automatically resets the original observation plane should drift occur.

- **Zeiss blues**
  The AxioCam family has a digital microscope camera for every application: the high-speed AxioCam HS, the highly sensitive AxioCam MRm for weak light intensities and the high-resolution AxioCam HRm.

The SteREO series offers maximum resolution even for large samples.

Digital cameras for microscopy: the AxioCam family fulfils every wish.

Modular and Expandable

AxioVision and ASSAYbuilder™ Drive Your Research Forward.

A useful addition: ASSAYbuilder™ expands the system software to include the functionality of High Content Analysis. In combination with the diverse AxioVision modules for acquisition and processing, ASSAYbuilder™ is software that's hard to beat in terms of performance and flexibility.

Acquisition has many facets

A precondition for successful HCA is image acquisition that captures every piece of image information precisely. With AxioVision, you can choose from a whole host of acquisition modules, depending on the application and type of sample.

- **Multichannel Fluorescence**
  Multichannel Fluorescence acquires up to 32 channels in fluorescence, transmitted-light and various contrast techniques.

- **Mark&Find**
  This module allows you to acquire images automatically at different positions on a specimen, e.g. from multiwell plates. Regardless of whether you are using standard formats, such as 96 or 384 well plates, or freely definable sizes, Mark&Find is a convenient way to automate image acquisition.

- **Time Lapse**
  This module enables you to generate image series over a period of time from which high content information on dynamic processes can be extracted.

- **Z-Stack**
  Using this module, you can generate image sequences from different focus planes as Z-stacks. This means that, if necessary, you obtain all the important information – and not just that from a single focus plane.

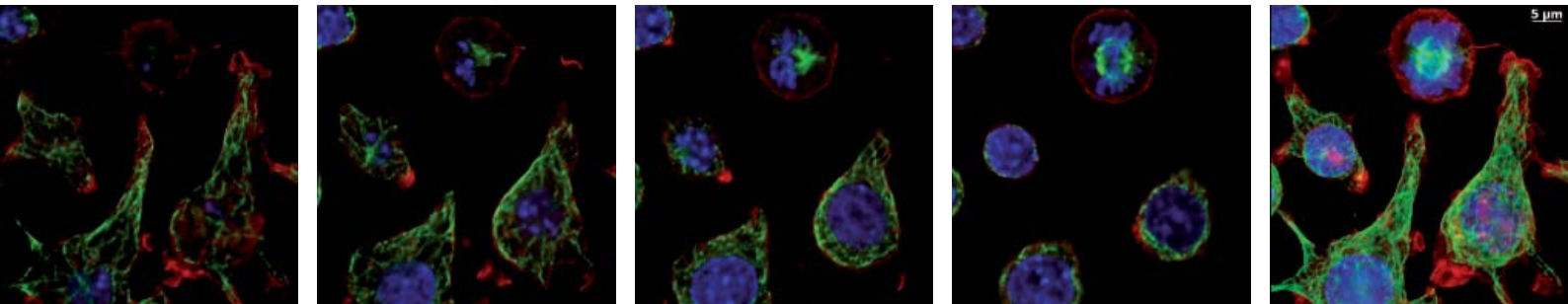
- **MosaiX**
  This module makes it possible to automatically scan large sample surfaces that extend beyond the camera's field of view. A seamless, virtual overall image is then generated from individual tiles.

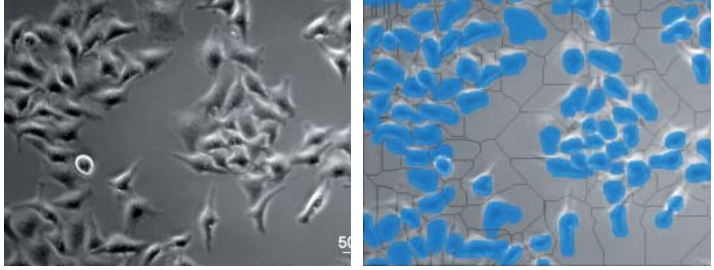
- **Autofocus**
  The Autofocus module calculates the optimal focus position in reflected-light, transmitted-light and fluorescence.

- **HDR (High Dynamic Range) Imaging**
  Imaging with HDR enhances the dynamic range acquired by the digital microscope camera. Very weak and very strong sample signals can therefore be acquired and analyzed simultaneously.

- **Extended Focus**
  In combination with your Z-stack images, you can use this module to extract the sharp details from every plane and generate a single image that is sharp across the entire thickness of the sample. This provides the perfect basis for obtaining high content information from just one image.

Four sections at various z-planes through a sample. On the far right is the Extended Focus image which contains the sharp information from all planes. Cells: N11 microglia with stained cell nucleus (blue), microtubules (green) and actin (red).
Dr. Birgit Kraus, University of Regensburg, Germany





Fluorescence labeling of cells is not always necessary to allow analysis using ASSAYbuilder™. Often ASSAYbuilder™ is also able to identify objects in bright-field or phase contrast images automatically. The example shows HeLa cells in a phase contrast image before (left) and after (right) object identification.

Image processing

AxioVision also offers modules in the area of image processing that effectively support HCA with ASSAYbuilder™ in terms of performance, sensitivity and precision.

- **Deconvolution**
  This module calculates and reassigns light below and above the focus plane back to its origin. This results in higher contrast, improved resolution and the recycling of valuable signals. Deconvolution is the prerequisite for 3D microscopy with widefield fluorescence images.

- **Widefield Multichannel Unmixing**
  Crosstalk between fluorescence signals in unwanted channels not only leads to unattractive images but also, in particular, to incorrect analysis results. Unmixing eliminates this crosstalk quantitatively, and consequently leads to a substantial improvement in the signal, better light-source efficiency and error-free data.

One analysis leads to another

AxioVision offers you a virtually unlimited range of options for analysis. A small selection clearly shows how AxioVision can help you place your research on a broader footing.

- **3D Measurement**
  3D Measurement offers you a wealth of opportunities for the interactive or automatic measurement of three-dimensional objects.

- **Inside4D**
  The Inside4D module allows you to observe your Z-stack images in space and time. With Inside4D you can visualize, scale and animate your images.

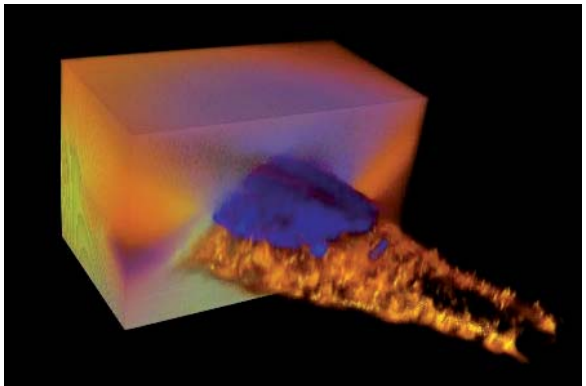
- **Tracking**
  This module analyzes the movement of cells and intracellular structures in your time lapse images both interactively and semi-automatically.

Automatically quicker: configuration with Commander

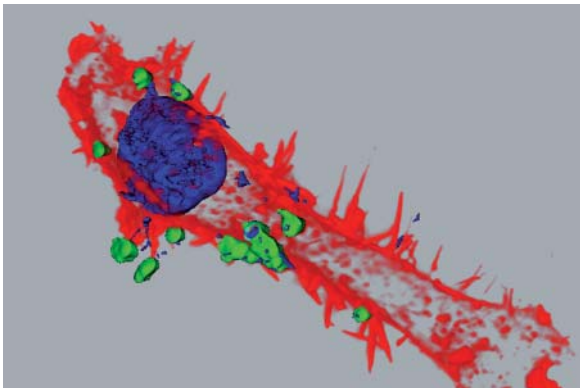
The advantages of Commander are that you can record the work steps to be performed one after the other, edit and refine this recording, set parameters and make everything available under a single command.

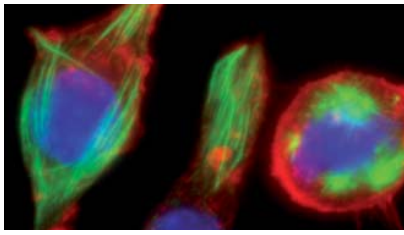
You will find even more modules for acquisition, processing and further analysis in our brochure "AxioVision. Perform to Perfection." and at www.zeiss.de/axiovision.

Volume data (Z-stack), acquired using widefield fluorescence. In the right-hand side of the image, light from outside the focus plane has been traced back to its point of origin using Deconvolution.
Cells: U138 astrocytes with stained cell nucleus (blue) and cytoplasm (orange).



3D reconstruction using the Inside4D module. BV2 microglia with stained cell nucleus (blue) and actin (red), as well as green S. aureus bacteria.
Dr. Birgit Kraus, University of Regensburg, Germany





ASSAYbuilder™

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