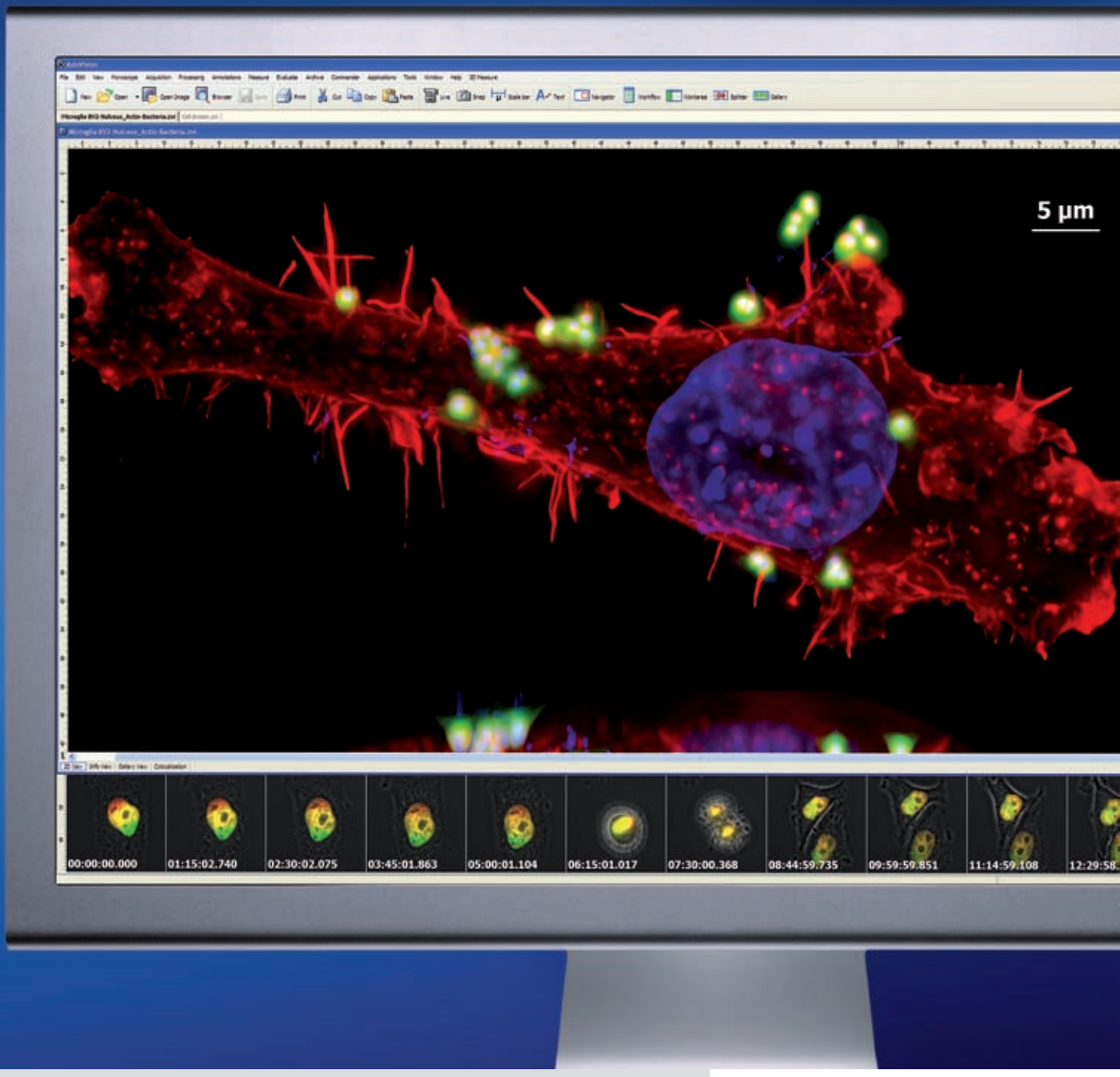
Cell Observer®

Living Cells



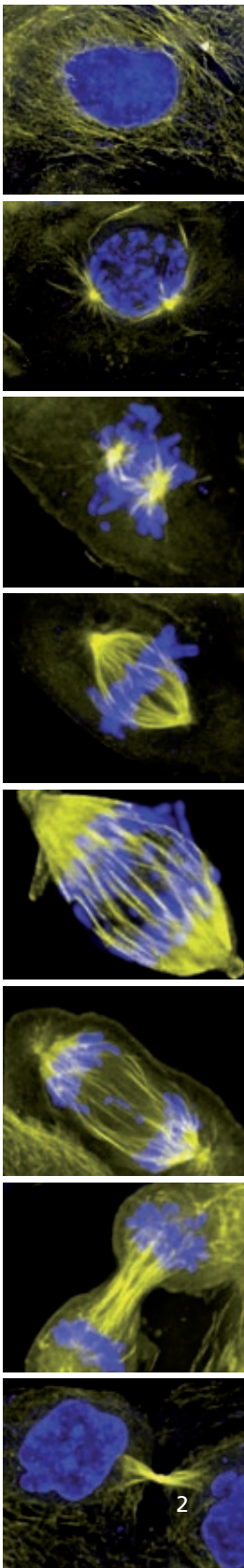
Observe and Analyze.

The Systems Approach for Imaging Living Cells and Organisms.

ZEISS

We make it visible.

Content

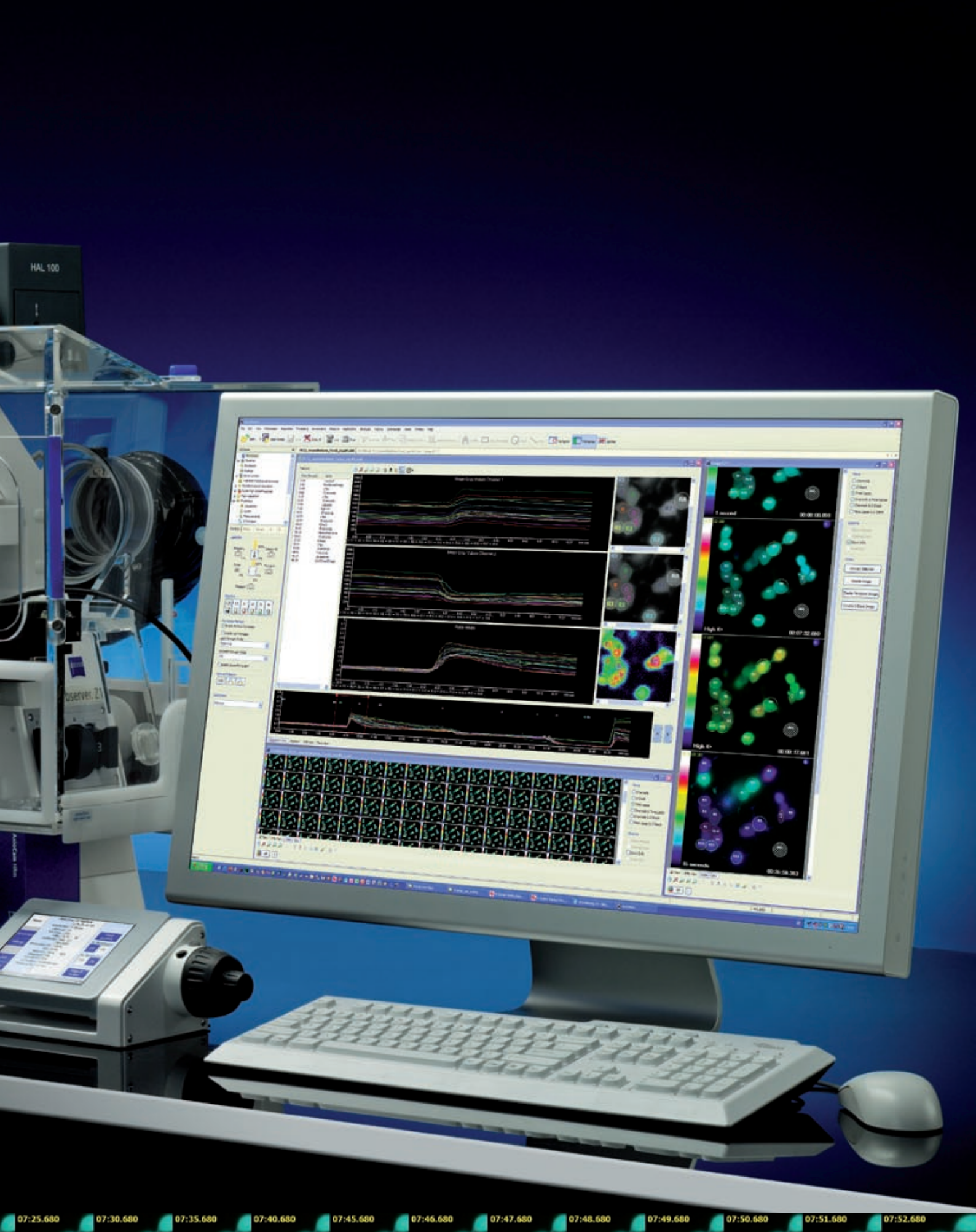


Multidimensional Acquisition with Cell Observer®

Multidimensional acquisition with Cell Observer® offers unprecedented flexibility and an unparalleled range of applications. Depending on your requirements, all acquisition methods, contrast techniques and workflows can be freely combined.

Dimensions	Function	Icon
Single Image Acquisition	2D image of a scene (color or monochrome, 8-16 bit)	
Time Lapse Imaging	A series of images of a scene at various time points	
Multichannel Acquisition	Images of the same scene using different contrast methods or fluorescence channels – combination of up to 32 channels	
Z-Stack Imaging	Images of the same scene including different focus planes; generation of an image volume by means of equidistant focus control	
Mark&Find	Acquisition of a large number of scenes by traveling to various sample positions with the help of a motorized stage	
MosaiX	Expansion of the scene beyond a camera's field of view; acquisition of adjacent tiles with the help of a motorized stage	
Smart Experiments	Configuration of heterogeneous experiments	


HXP 120
CO2 Module S
Heating Unit XL S
ZEISS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Cell Observer® Offers Many Dimensions.

The Crucial One Is Performance.

Modern research in the field of life science calls for powerful Imaging Systems that can be used flexibly, are easy to operate and are equipped with a whole host of functionalities. A convincing example of such a system is Cell Observer® from Carl Zeiss.

In focus: cutting-edge technology for research with cell cultures

An increasing number of tasks in medicine and biology rely on research with human and animal cell cultures. Vesicle transport, reporter assays, gene expression and regulation or viral expression: these and other applications involve working at an extremely high technological level. At the same time, the required sample throughput is increasing – a considerable challenge for the corresponding Imaging Systems and their components.

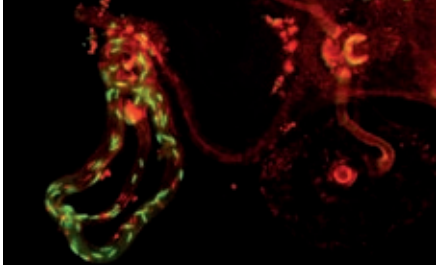
After all, to be able to observe cells under biologically relevant conditions, all components need to be seamlessly integrated. A task to which we at Carl Zeiss have been devoting all of our expertise and commitment since the introduction of Cell Observer® in 2000. The result: the optimized Cell Observer® system, which excels thanks to its impressive range of applications, level of integration and ease of operation.

A forward-looking systems approach for all research tasks

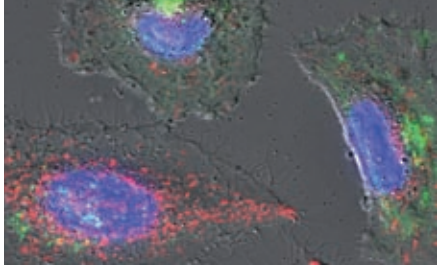
What makes the latest Cell Observer® generation stand out in particular is the system philosophy: interaction between extremely powerful components that support the workflow in the best possible way. Depending on your requirements, this results in a wide range of possible configurations, which can be expanded modularly at any time. The AxioVision software platform functions as a central control point. In addition to component control, a core function offered by AxioVision is the possibility to freely combine all image acquisition dimensions. All requirements in the field of Live Cell Imaging are met by Cell Observer®, from short time lapse imaging at very high speed to long-term experiments lasting several days and involving a number of different fluorescence channels, z-planes and positions. Cell Observer® consists of the following components:

- Axio Observer and Axio Imager research microscopes
- Specially developed objectives for Live Cell Imaging
- Incubation with control of temperature, CO_2, humidity or O_2
- Cameras of the AxioCam family and selected EM CCD cameras from third-party manufacturers
- State-of-the-art light sources and filter sets
- High-speed mode with streaming technology (Cell Observer® HS (High Speed))
- Accessories such as fast shutters and precise piezo focusing
- Components for additional applications (ApoTome for optical sectioning, TIRF for observing membrane processes)

These components combine to give you outstanding performance because they have been carefully integrated into a system. For you this means that, from simple observation and documentation to the quantitative analysis of slow and rapid processes, Cell Observer® guarantees a wide range of applications, a complete workflow, and convenient operation.



Gonads of female flies (red) with sperm cells (green)
Dr. Scott Pitnick, Syracuse University, New York, USA



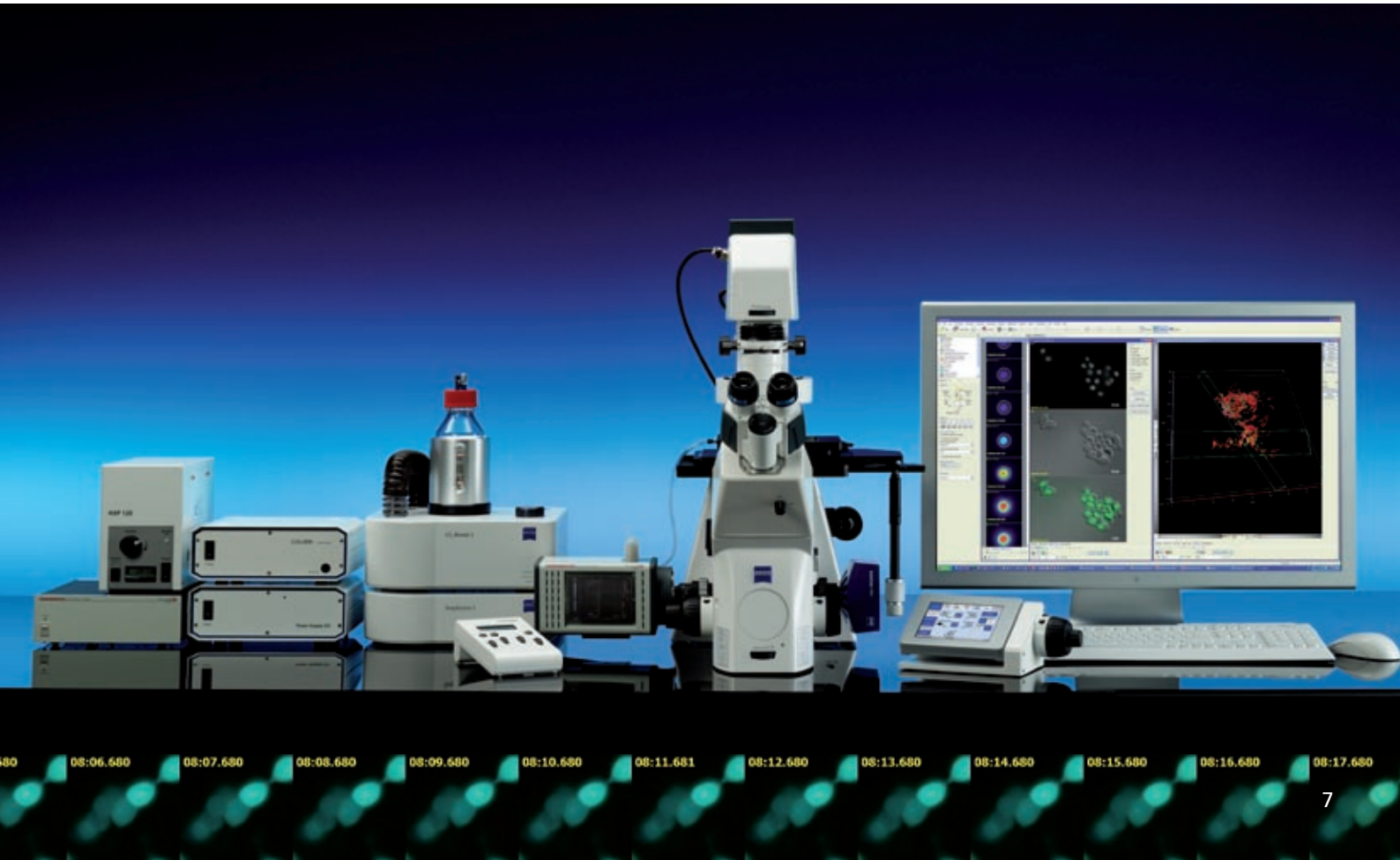
HeLa cells, reggie-GFP expression, stained using MitoTracker Red and HOECHST 33342
Prof. Claudia Stürmer, University of Konstanz, Germany

A secure and future-proof investment

The possibility of performing experiments rapidly, operating even complex technology simply and having precisely the needed functionality at your disposal – because of these demands, many users are keen to find a complete solution. Boxed solutions often perform only specific applications sufficiently well. As a rule, however, significant compromises have to be accepted when it comes to expandability, flexibility and optical quality.

Scientific discovery, though, is unpredictable by its very nature. Where will my research lead me tomorrow? Can I be sure that my applications will not change? How secure will my investment be in the future? If you want to prepare yourself today for the research tasks of tomorrow, you put your faith in a future-proof platform. In a system that grows with the tasks to be carried out, that has been developed to offer maximum flexibility and that is open to future developments. A system that has been designed to offer convenient workflows in the most complex of applications and has been realized as a fully integrated research platform for cell observation and cell analysis – Cell Observer® from Carl Zeiss.

Cell Observer® HS with Hamamatsu ImageEM and AxioCam HRm, Incubator PM S1 with Heating Insert P S1



Every Camera Has Its Strengths.

High Resolution or High Speed? The Application Decides.

Maximum speed, maximum sensitivity or both: with Cell Observer® you can use exactly the right camera for each application. Either a CCD camera from the Carl Zeiss AxioCam family or the most powerful models offered by third-party manufacturers.

The AxioCam family – established in science and research

Performance features that speak for themselves: the third generation of Peltier-cooled digital cameras from Carl Zeiss stand out thanks to their high sensitivity, high dynamic range as well as maximum resolution and image quality. Live cell applications with Cell Observer® require the highest possible sensitivity and resolution. Therefore monochrome cameras are used almost exclusively.

In addition to the tried-and-tested AxioCam MRm, we can particularly recommend AxioCam HRm, a camera which can be employed universally. This powerful camera offers fast microscanning modes, allowing images to be acquired with a resolution of up to 12 megapixels. With a signal to noise ratio of at least 1:2500 in 14 bit the HRm is the AxioCam model with the highest sensitivity.

The specialist for maximum speed is AxioCam HSm. Its frame rates of up to 360 images per second (with Binning 5x5 and ROI) – roughly 15 times faster than with analog video cameras – offer you maximum performance. Whichever AxioCam you opt for, it will be seamlessly integrated into the high speed concept of Cell Observer®.

ImageEM camera from Hamamatsu

For color images, e.g. to determine the coloration of certain microorganisms for classification purposes, it is of course also possible to use any of AxioCam color cameras.

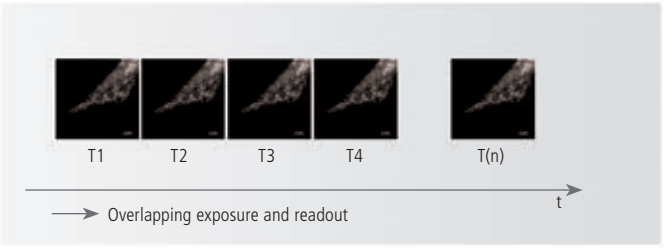
AxioCam HRm, the camera with the highest sensitivity from Carl Zeiss

EM CCD cameras: the photon collectors for the weakest signals

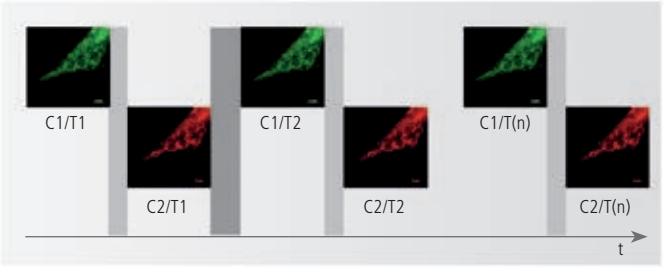
For the most challenging applications you can also easily integrate special cameras from third-party manufacturers into Cell Observer®. If, for example, you want to detect individual fluorescence molecules or acquire images of rapidly moving but very weakly stained cell components, cameras can be employed which can amplify the signal before it is being digitized. This technique is known as Electron Multiplication CCD, or EM CCD for short.

Cell Observer® offers efficient compatibility with the models from the companies Hamamatsu and Photometrics. For example, the EM CCD camera ImageEM (Hamamatsu 9100-13) offers maximum sensitivity with a resolution of 512 x 512 pixels. This ensures that Cell Observer® collects everything, right down to the last photon.

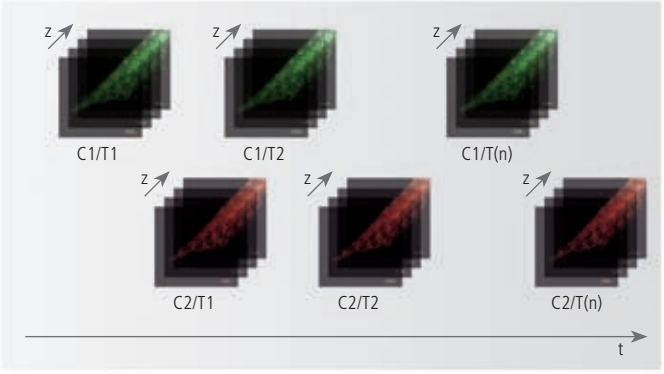
Frame Rates for Extremely High Acquisition Speeds



Dimensions			Frames/second	
Channels	z-planes	Binning/ROI	AxioCam HSm	AxioCam MRm
1	1	no	61	14
		yes	186	42



Dimensions			Frames/second	
Channels	z-planes	Binning/ROI	AxioCam HSm	AxioCam MRm
2	1	no	31	7
		yes	94	29



Dimensions			Frames/second	
Channels	z-planes	Binning/ROI	AxioCam HSm	AxioCam MRm
2	20	no	24 (= 1.7 sec./ time point)	7 (= 5.6 sec./ time point)
		yes	40 (= 1.0 sec./ time point)	25 (= 1.6 sec./ time point)

If Cell Observer® is operated in high speed mode, the camera will be used at maximum speed. The data are written directly to the hard drive as a stream. If the camera is the only component, it can be operated in so-called "overlapping mode" (top figure). The maximum speeds that can then be achieved depend exclusively on the selected resolution and ROI. Binning increases sensitivity and speed still further. The highest acquisition rates in this mode of operation are achieved using AxioCam HSm.

When other components, such as light sources, are used (middle figure), precise synchronization of the camera, light source and PC is required to achieve high acquisition rates. In this case the achievable acquisition rates are approximately halved, depending on the settings used.

For acquisition in the third dimension (third figure) a fast piezo focusing unit is employed. The maximum focusing speed is limited to 30 planes per second to achieve sufficiently high positioning accuracy. Under these conditions the camera is no longer the rate-limiting factor. In this case use of the higher resolution and more sensitive AxioCam MRm in combination with binning makes more sense.

Sensitivity: the Interaction of All Components Determines Overall Efficiency.

Various components play a role in the excitation and detection of the frequently used live cell dye GFP.

The table below compares a selection of such components and shows examples of potential efficiency increases.

Type	Component A	Component B	Approximate efficiency increase
Filter	Filter set 38	Filter set 38 HE (High Efficiency)	80%
Numerical aperture	Plan-NEOFLUAR 40x/0.75	Plan-NEOFLUAR 40x/1.3	800%
Transmission 488/515 nm	LD A-Plan 40x/0.50 Ph2	EC Plan-NEOFLUAR 40x/0.75	15%
Quantum efficiency	Sony ICX-285 (e. g. as in AxioCam MRm): approx. 65%	Back Thinned, Back Illuminated CCD (512 x 512): approx. 95%	46%

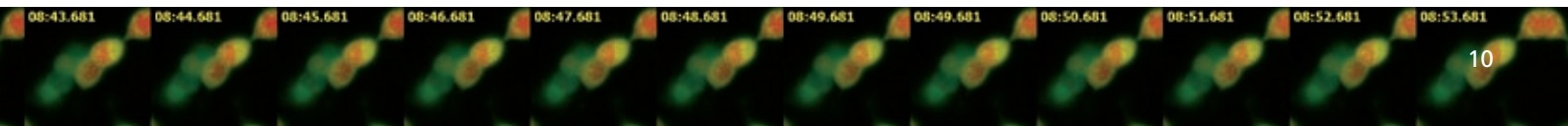
Other components also contribute to overall efficiency. However, their effect depends on too many factors to allow simple quantification. A few examples are provided below:

- **Illumination source**
  The efficiency of the Colibri LED illumination system (LED 470 nm) is significantly higher for GFP than that of HBO illumination. The latter has light components outside the desired spectrum and is also fairly weak in the desired wavelength range from 470 nm to 490 nm.

- **Camera**
  Besides basic camera parameters such as quantum efficiency, many other parameters influence the end result to a greater or lesser extent. If, for example, binning is used, resolution is sacrificed in favor of sensitivity. Options such as EM CCD sensors can bring about an additional increase in sensitivity within a relatively narrow range of applications.

- **Objective transmission**
  If other dyes are used, e.g. in the UV or infrared range, transmission differences may in some cases be considerably greater than indicated in the table.

Without detracting from the importance of the choice of camera, it is clear that other components can have a much greater impact on the overall efficiency of a fluorescence system. The careful selection of all components, particularly in terms of the cost/benefit aspect, is therefore crucial. Online databases can help with this assessment (see http://www.zeiss.de/micro-shop, under Tools ⟶ Camera-Assistant, Filter-Assistant or Objective-Assistant).



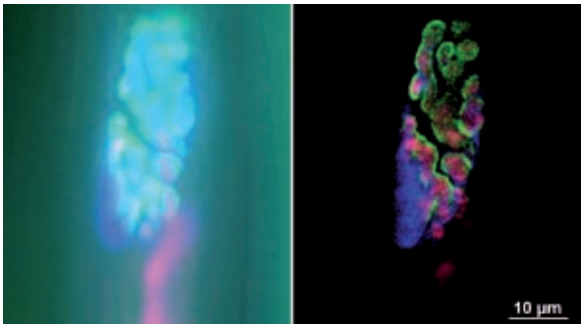
A High-end System for Universal Use.

With Rapid Switching to Complex Applications Such as TIRF.

Cell Observer® is a proven leader in its field. From routine tasks and complex live cell applications through to 3D Imaging, but also in specific applications such as TIRF. With extremely convenient operation and without the need for laborious modifications.

Razor-sharp optical sections: ApoTome

The research platform as 3D workstation: with the ApoTome slider module you can generate optical sections of your fluorescence samples with extremely high resolution using Cell Observer®. ApoTome works using the principle of structured illumination. And as you move the slider to the park position, all the possibilities offered by Cell Observer®, including those in high-speed mode, are immediately available to you once again.



Neuromuscular junctions, left shows widefield, right shows ApoTome. Sample: Wes Thompson, USA

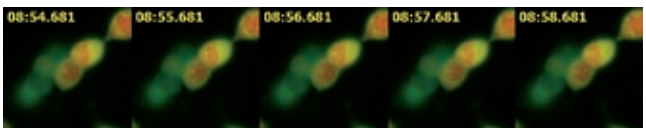
For maximum resolution in the evanescent field: TIRF

The Laser TIRF slider from Carl Zeiss allows to visualize the most rapid membrane processes with very weak signals using extremely short exposure times. And with the best contrast, directly from the interface between the cells and cover slip. Again, switching the acquisition mode to TIRF is incredibly easy. In addition to the laser module and TIRF slider, a safety kit to ensure compliance with laser safety regulations is also integrated.

The universal system with intelligent accessories

Flexibility with regard to your applications calls for flexibility in the area of accessories. A wide range of accessories is available for Cell Observer®, such as excitation and emission filter wheels or fast external shutters. With Cell Observer® HS the Uniblitz shutter is inserted into the transmitted-light path to allow ultra-fast switching between transmitted-light and fluorescence. Complex applications combining fluorescence with phase contrast or brightfield can be carried out quickly and reliably.

ApoTome (top) and TIRF (bottom) sliders transform Cell Observer® into a system for generating optical sections.



Cells Need the Perfect Climate.

And Perfectly Harmonized Components.

However varied your incubation applications may be, they are matched by the possible Cell Observer® configurations. From simple heating to the detailed control of all key environmental parameters.

An entire systems approach for incubation

Besides sophisticated technology, successful incubation calls for a great deal of expertise and experience. What is the best position for the temperature sensor? When is it important to seal the system as perfectly as possible and when is it better to use an open system? Such details often determine whether extremely sensitive cells can also be successfully incubated on a microscope. Carl Zeiss has been developing incubation systems for more than 15 years and is continuing to push the development in this segment. From simple heating or cooling through to incubation solutions for applications with ApoTome or TIRF, Cell Observer® combines precision with a wide temperature range and seamless integration, also into the AxioVision software platform. All in all this means that you have a huge range of applications at your disposal. And a system that grows economically with the tasks to be carried out.

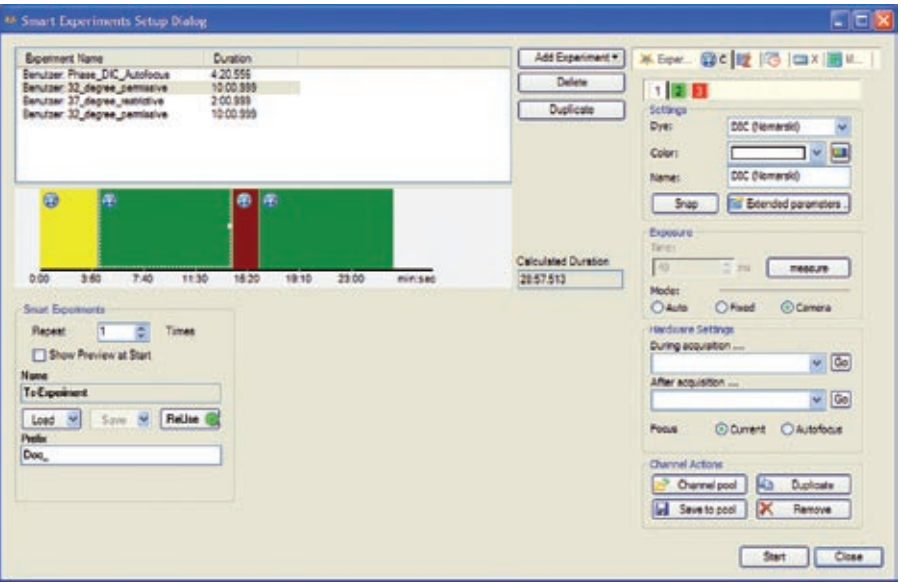
Gene expression experiment over 16 hours (DIC, YFP, mRFP). Focus stability is achieved after only 5 minutes.

Innovative: stacking modules

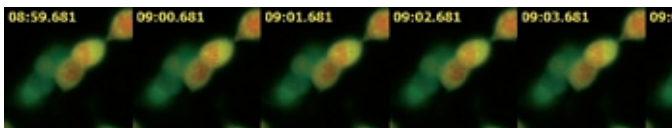
Space-saving, economical and exclusive to Carl Zeiss: the stacking concept for the control modules used to control the components. All components are controlled centrally by TempModule S1 connected to the PC. This allows easy upgrades or retrofits.

Unique: best possible imitation of In-vivo conditions

During incubation with Cell Observer® cultivation conditions are created that simulate the environment in the living organism in the best possible way: humidity, the correct temperature, CO_2-control for the correct pH value and physiological O_2 concentrations. The Incubator XL encases all the important opto-mechanical components of the beam path and stabilizes them thermally. This effectively prevents fluctuations in the ambient temperature and the inevitable effects these would have on focus stability. Consequently, even experiments lasting several days can be performed without focus drift and without damage to the cells.



Smart Experiments: flexible control of all incubation parameters during complex time lapse experiments.



The wide range of available incubation components, as well as the different combination options and configurations, can be found in our brochure "Cells Need the Perfect Climate" and at www.zeiss.de/incubation.

Cutting-edge microscopy:
Axio Observer and Axio Imager

In the field of research, the Axio Observer and Axio Imager have proven themselves as research microscopes with the best fluorescence and optical performance. For applications e.g. in developmental biology the upright Axio Imager microscope is ideal. To observe living cell cultures the motorized Z1 model of the inverted Axio Observer microscope is usually employed. Both microscopes offer unrivaled performance in all key areas.

- Improved light path with optimum correction
- Convenient operating concept
- Fast and vibration-free motorization
- Flexible and adaptable electronics concept
- Best illumination homogeneity
- Contrast-optimized optical components
- Unparalleled stability, expandability and user-friendliness

Irrespective of which version is required for your application, both microscopes will give you outstanding results, from simple observation to high-end applications.

In a class of their own: the objectives

Carl Zeiss objectives such as EC Plan-NEOFLUAR and Plan-APOCHROMAT are the eyes of Cell Observer®. They have been developed to carry out the different tasks associated with Live Cell Imaging in an outstanding way. LCI Plan-NEOFLUAR 63x/1.3 Imm. Korr. and LD-LCI Plan-APOCHROMAT 25x/0.8 Imm. Korr. have been specially designed for Live Cell Imaging. Thanks to the following properties they are ideally suited to the conditions of cell and tissue cultures:

- Ideal for samples in aqueous environments
- Excellent transmission properties
- Highest possible numerical aperture
- Correction ring to avoid spherical aberration: for 23-37°C
- Immersion: water, glycerol or oil (25x only)
- Color-corrected for the spectral range of the vast majority of living-cell dyes used today

Selection of objectives designed specifically for Cell Observer® applications and offering very high working distances (left) or multi-immersion at 25-37°C (right).

The research microscopes Axio Observer and Axio Imager

Bright, Fast and Gentle: the Light Sources.

Life Science applications place extremely high demands on light sources. High light intensity, stable mechanics or electronics, long life and simple operation are essential. Exactly these performance criteria are fulfilled by Colibri, for example.

Colibri, the innovative LED fluorescence light source – fast, flexible and without mechanical switching.

The standard: white light sources

The selective staining of certain structures in cells and tissues using fluorescent dyes calls for a flexible source of excitation. The simplest solution: a white light source, either in the form of a Mercury or Xenon lamp. With Cell Observer® you can choose between directly coupled lamps, e.g. a HBO103 or a light-guide-coupled metal-halide lamp such as the HXP 120.

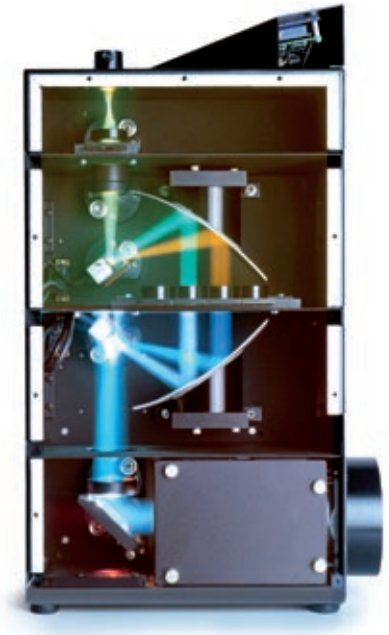
New light in fluorescence: Colibri from Carl Zeiss

High-performance LEDs (Light Emitting Diodes) instead of a white light source – thanks to this innovation it is possible, with Colibri, to take full advantage of the many benefits offered by LEDs for fluorescence applications. Each LED delivers a precisely defined range of the spectrum. No undesired light is generated, which means that there is also no need to suppress it. The overall result is that you are able to benefit from extremely high-contrast images which are ideally suited to quantitative image analysis.

- Intensity can be tailored precisely to the sample
- No intensity fluctuations like with arc lamps
- Switchable in the microsecond range without mechanical shutters
- Long life, cost effective and modular design

Switch in 1.7 ms: Sutter Lambda DG-4

The universally applicable light spectrum makes the Lambda DG-4 Xenon lamp from Sutter the ideal light source for applications in the UV range, for example, such as Calcium Imaging using the dye Fura-2. It can control up to four filter positions and switch between them in 1.7 ms. The light is coupled into the microscope by means of a light guide.

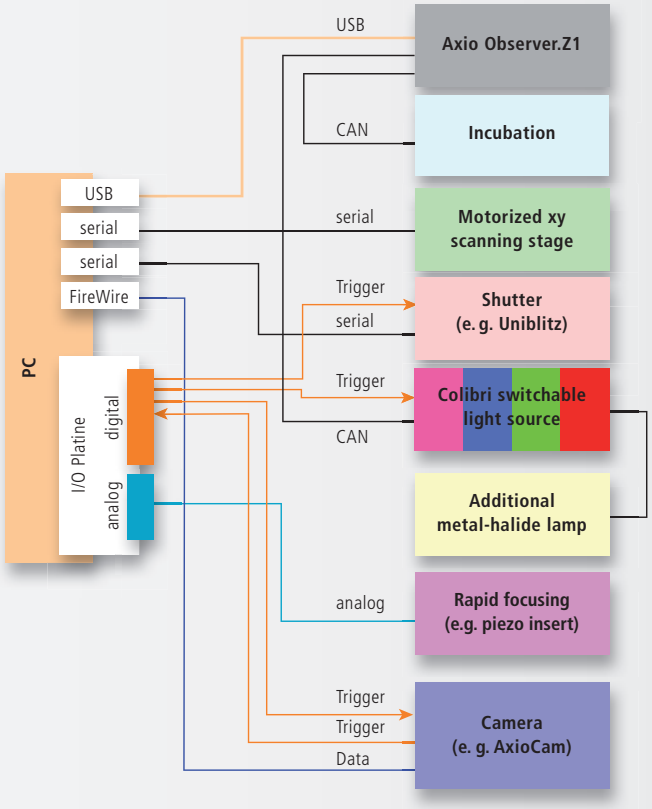


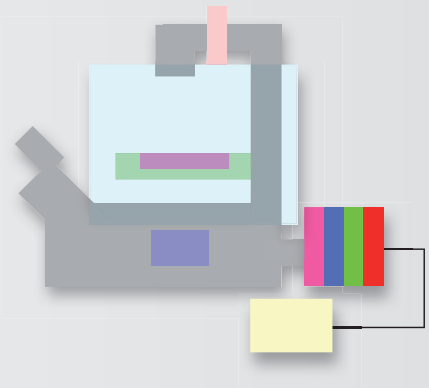
The Lambda DG-4 Xenon light source allows rapid switching between 4 wavelengths using two small mirrors. For this to work, a combination of single wavelength excitation filters and high efficient multibandpass dichroics and emission filters are needed. Examples are filtersets 54 (CFP, YFP, HcRed), 55 (CFP, YFP) and 56 (GFP as well as most of the red fluorescent proteins such as DsRed) and the range is growing continually.

The perfect addition: multi-band pass filter sets

Cell Observer® can be equipped with a whole range of multi-band pass beam-splitter and emission filters. The filter sets have been adapted to the Colibri and Lambda DG-4 light sources and offer maximum transmission and extremely steep slopes.

System Diagram for Cell Observer® HS with Colibri





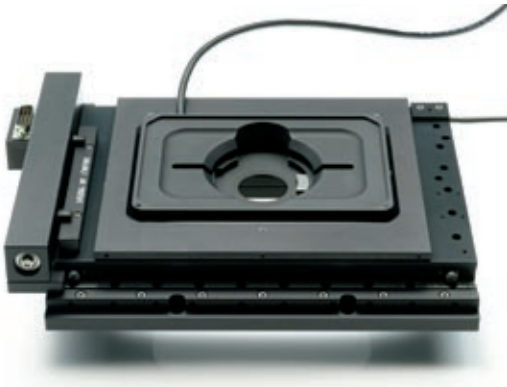
Overview of the component circuitry for a sample Cell Observer® HS configuration using the Colibri as a light source. All time-critical components are controlled directly via electrical signals (analog or TTL). This avoids time-consuming software commands and allows the maximum speed which can be technically achieved.

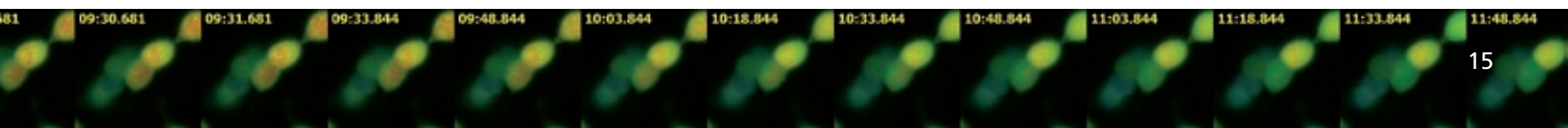
Fast and precise: motorized stages and piezo focusing

Cell Observer® also allows a choice of several motorized stage models that will make it easy for you to achieve a higher sample throughput and high-resolution images. The fast and economical scanning stage (432031-0000-000) with CAN bus control is ideal for magnifications up to 40x. It is controlled directly via the Axio Observer.Z1 microscope. The tried-and-tested stepper-motor stage (432033-0000-000) is also recommended for higher magnifications. Both of these stages can be used for multiwell plates. If you want to document rapid processes in 3D, you need the highest possible focusing accuracy. With Cell Observer®, piezo based focusing devices have been designated for this purpose. These deliver precise three-dimensional images of extremely rapid movements in multiple z-planes. The ideal solution: the high-end scanning stage (000000-0473-167) with piezo focusing top plate. This has a z-traveling range of 100 µm with a minimum step size of 1.5 nm. Up to 30 focus steps can be executed per second. The advantage of the piezo insert is that all objective positions and all contrast techniques are supported.

In combination with Incubator XL, the specially adapted Heating Insert P S1; compact and the CO_2-cover, even incubation is possible.

Scanning stage with piezo insert and Heating Insert P S1; compact: Fast piezo focusing combined with precise xy positioning, ideal conditions for e.g. Petri dishes with glass bottom or Lab-Tek™ chambers.





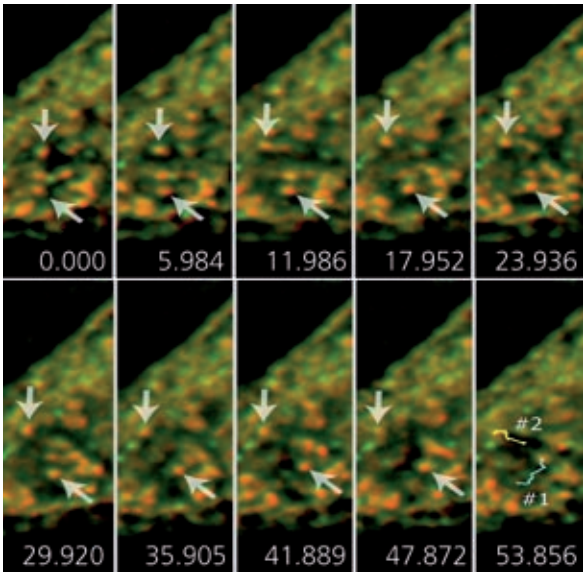
When Researching Life, Every Second Counts.

And Sometimes Every One of Your 1200 Images.

To allow observation of the most rapid of processes, a system must be able to do one thing above all else: to be faster with highest reproducibility.

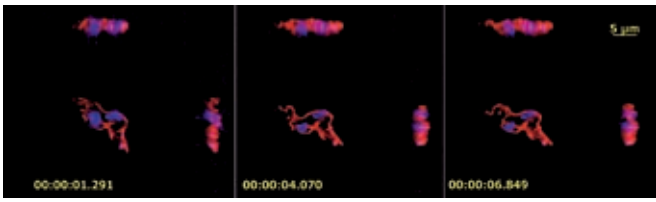
Tracking – analysis of vesicle movement in living cells

Several parameters are important when it comes to analyzing dynamic processes in cell cultures. Therefore the AxioVision Tracking module allows measuring the following: distance, speed and the ratio of straight distance to total distance, i.e. the measure of tortuosity. The figure shows an ROI from a time lapse image comprising 30 time points in various planes, along with the associated measurement values (table).



Track 1	Start Frame	End Frame	Time [sec]	Total Distance [µm]	Staight Distance [µm]	Mean Velocity [µm/sec]	Tortuosity [total/direct distance]
1	1	30	57.85	4.13	2.05	0.07	2.02
2	1	30	57.85	4.65	1.76	0.08	2.64

HeLa cells, Ergic53-GFP and signal sequence DsRed, time lapse Z-stack image, processed using 3D Deconvolution. Every third image from the time lapse series containing a total of 30 time points at z-plane #8 and table of measurement values. Measurement of two particles in DsRed channel with the Tracking module (see table for analysis). Houchaima Ben Tekaya, Biozentrum, University of Basel, Switzerland.



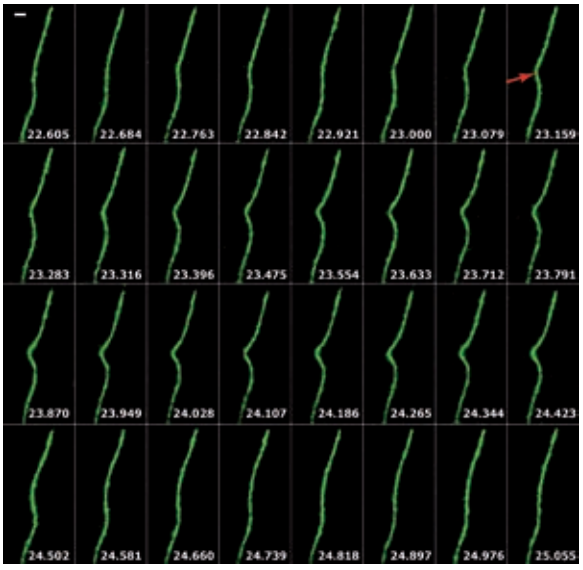
Living trypanosomes during cell division. DAPI staining shows the cell nucleus and mitochondrial DNA. MitoTracker Red staining highlights mitochondrial membranes. The Z-stack time lapse image was exported as a sectional view (xy, xz and yz direction) following Deconvolution. Cells: Dr. Torsten Ochsenreiter, USA

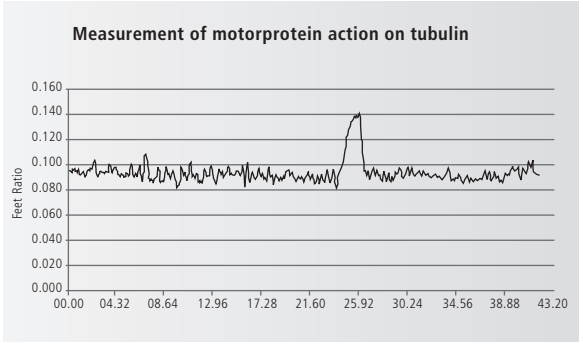
Precise Z-stack images of living trypanosomes in two channels

Living trypanosomes move at very high speed with the help of an undulating membrane. The example shows images of a cell in the process of division in two channels (MitoTracker Red and DAPI) with 30 z-planes per time point. A time point is acquired in 2.7 seconds. The image series consists of 20 image stacks with a total of 1200 individual images.

Maximum Performance Requires Maximum Flexibility.

The possibility of freely combining all image acquisition dimensions with Cell Observer® opens up a wide range of even complex applications. All with a convenient, universal workflow, maximum precision and uncompromised reproducibility.



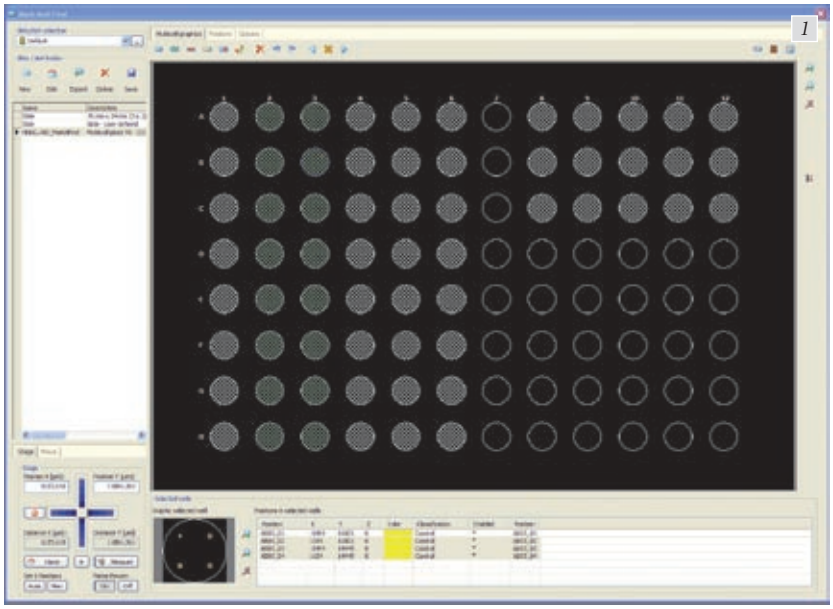


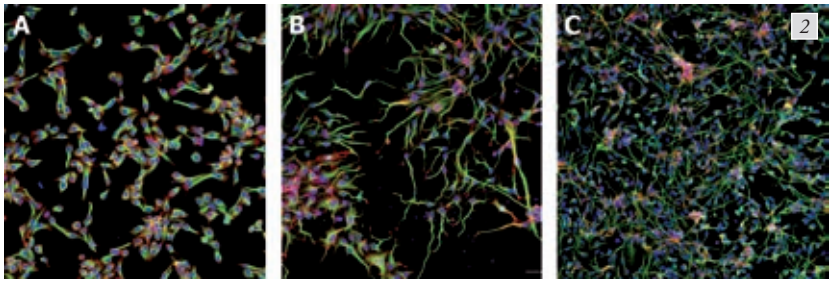
Sample: Gijsje Koenderink and Marina Soares de Silva, FOM Institute for Atomic and Molecular Physics, Amsterdam, Netherlands.

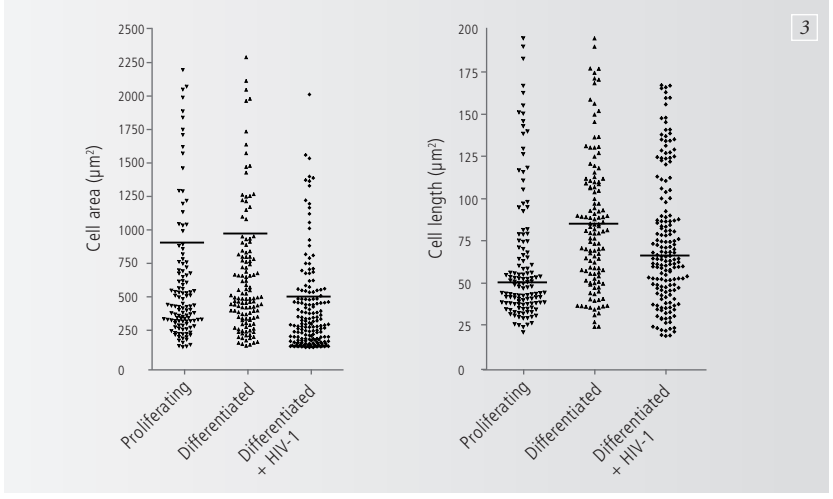
Time lapse imaging of protein processes at the molecular level: tubulin measurement

With Cell Observer® it is also possible to capture and quantify processes at the molecular level. The time lapse image presented shows how the motor protein myosin II bends a microtubulin fiber located within a network of actin fibers.

This rapid event was acquired at a framerate of 27 images per second. A red arrow marks the place where the bending occurs. The action of the motor protein is clearly visible. To quantify the event, the ratio between the smallest and largest feret was presented. An increase in the feret ratio for a period of around 3 seconds can be seen.







1) Mark&Find software dialog

2) Multichannel images: 3-channel images of the neural stem-cell line HNSC.100 using the AxioVision modules Multichannel Fluorescence, Mark&Find and Autofocus. Scale bar 50 µm. A: proliferating cells (control), B: cells following treatment with differentiation factors, C: differentiation and simultaneous HIV-1 infection. The differentiation of the stem cells into neurons (B) can be clearly seen, as can additional effects resulting from the HIV infection (C).
Images used with the kind permission of Prof. Ruth Brack-Werner, GSF Neuherberg, Germany (Rothenaigner et al, AIDS 2007, 21:2271-2281).

3) Charts with ASSAYbuilder measurement results

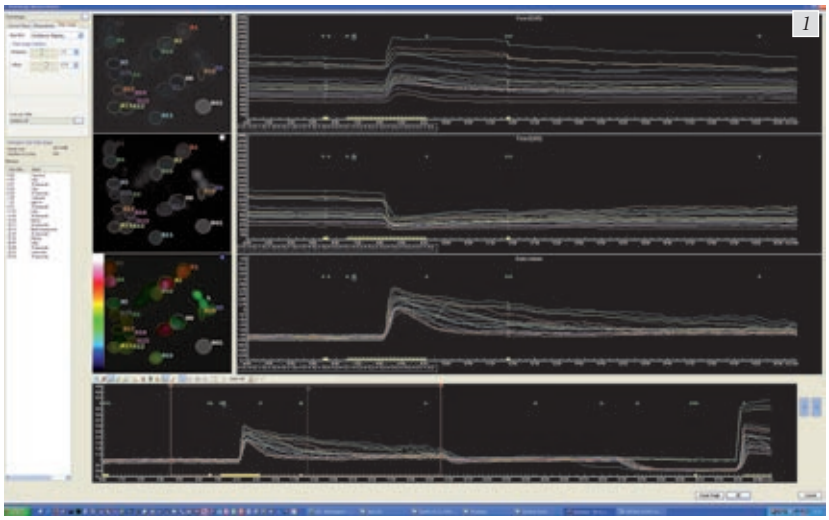
For reproducible results: Mark&Find

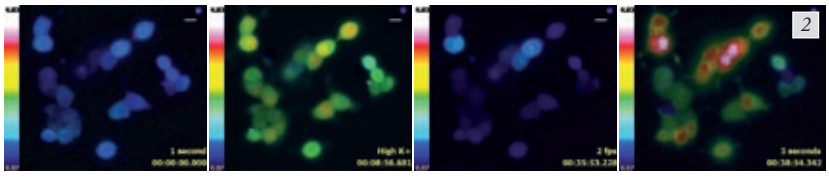
To increase the statistical significance, repeating experiments or increasing the number of images acquired is common practice in Live Cell Imaging. This requires a system offering extremely high precision. The AxioVision Mark&Find module was developed with this in mind. It delivers hundreds of images from multiwell plates or Lab-Tek™ chamber slides quickly and very conveniently. Thanks to a well-thought-out software dialog, even complex sample scenarios can be configured quickly.

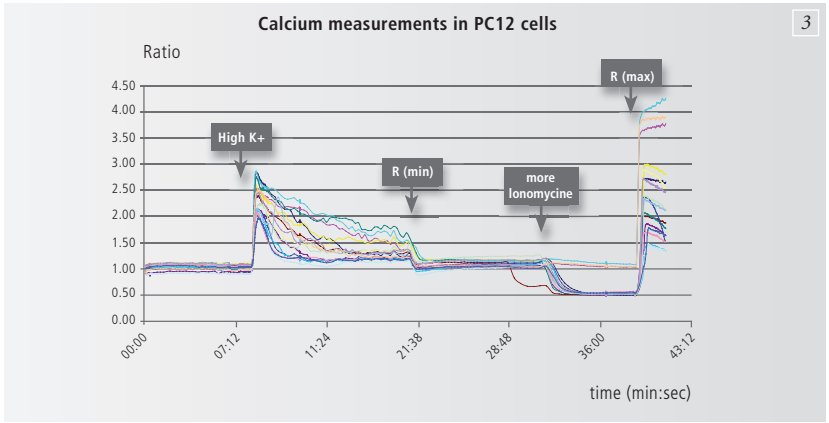
In the experiment shown the influence of HIV-1 infection on the differentiation of human neural stem cells was investigated. The treated cells were triple-stained and analyzed automatically following the acquisition of three-channel images.

Cell based high content analysis using ASSAYbuilder

Reliable statements about such effects cannot be made solely on the basis of the visual impression. For this reason quantitative image analysis is important. Here both the area and length of individual cells were measured using the AxioVision ASSAYbuilder module. Although a difference between the three populations can also be identified by looking at the images, modulation of differentiation due to the HIV infection can only be demonstrated convincingly by means of quantitative analysis (see charts).







1) A convenient dialog allows the acquired sequence to be analyzed. Markers set during acquisition make it possible to navigate rapidly within the experiment. A range of different display and calculation methods exist.

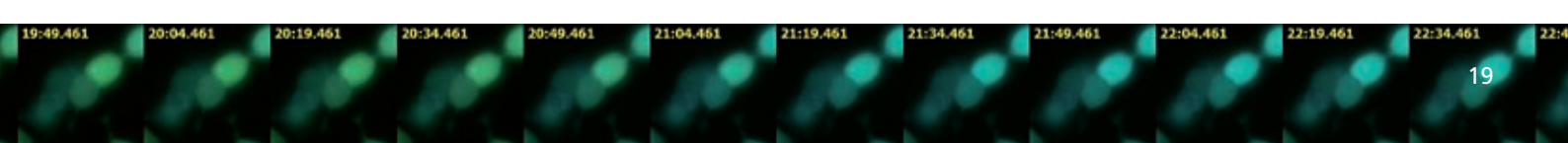
2) Murine PC12 neuroblastoma cells, stained using Fura-2. The figure shows four time points from the experiment in the form of a ratio image, calculated by dividing the 340 nm channel image by the 380 nm channel. The increase in calcium (2nd image) can be clearly seen from the change in color. In the 3rd image it is possible to see the minimum calcium concentration, resulting from ionomycin treatment, and in the last image, the maximum calcium increase in the presence of a high calcium buffer. These two values R(min) and R(max) can be used to calibrate the system. The experiment was performed using cells from Dr. Roberto Levi in the laboratory of Dr. Randi Silver, Cornell-Weill University, New York, USA

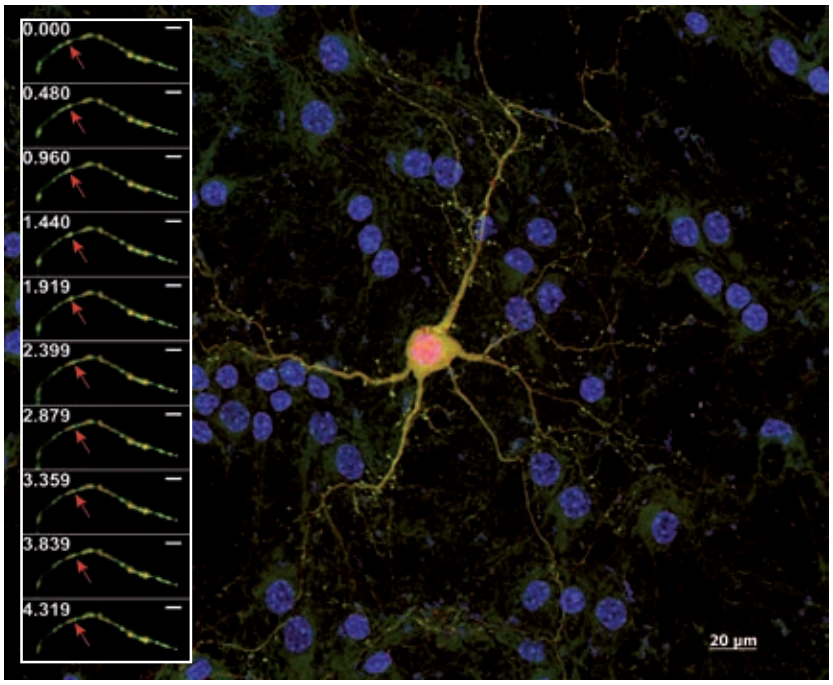
3) Analysis of the PC12 Fura experiment: the Physiology module generates a range of output documents, including the measurement value table containing the data, shown here in the form of a diagram.

Physiology: ion concentration change and analysis

Using the AxioVision Physiology module it is possible to analyze ion concentration changes in the cell. A range of fluorescent dyes exist that change their spectral properties when they bind to ions such as calcium. An example of such a dye that is frequently used is Fura-2. The pH-sensitive dye BCECF also allows intracellular pH-value measurements to be performed. A number of conditions need to be observed here:

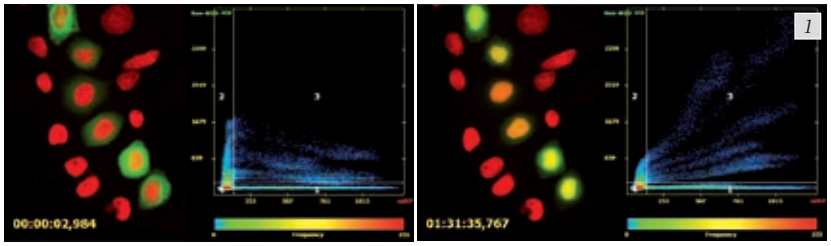
- Imaging must take place under quantitative conditions
- Frequently, images at two wavelengths are ratiometrically processed to avoid artifacts resulting from bleaching or changes in focus
- Many physiological processes occur rapidly, which means that you need to use components which can be switched quickly
- Data evaluation and charting takes place during acquisition in order to allow active steering of the experiment

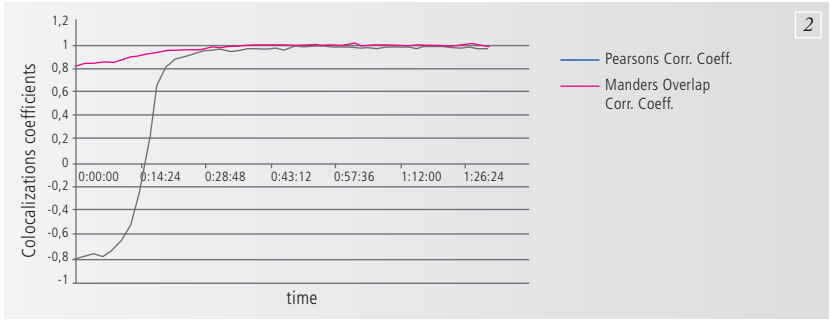




Combinatorics

Samples and images: K. Czöndör and K. Schlett, Eötvös Lorand University, Dept. Physiology and Neurobiology; Budapest, Hungary





Colocalization

1) Histone-2B-expressing HeLa cells, transfected with a cytoplasmically localized mutant of the HIV protein Rev: following treatment with a nuclear export inhibitor this Rev mutant relocalizes to the nucleus. The figure shows two time points, before and after treatment. The scatterplot clearly shows the increase in the colocalization of histone-RFP and Rev-GFP.
Cell cultures: Prof. Ruth Brack-Werner, GSF Neuherberg, Germany

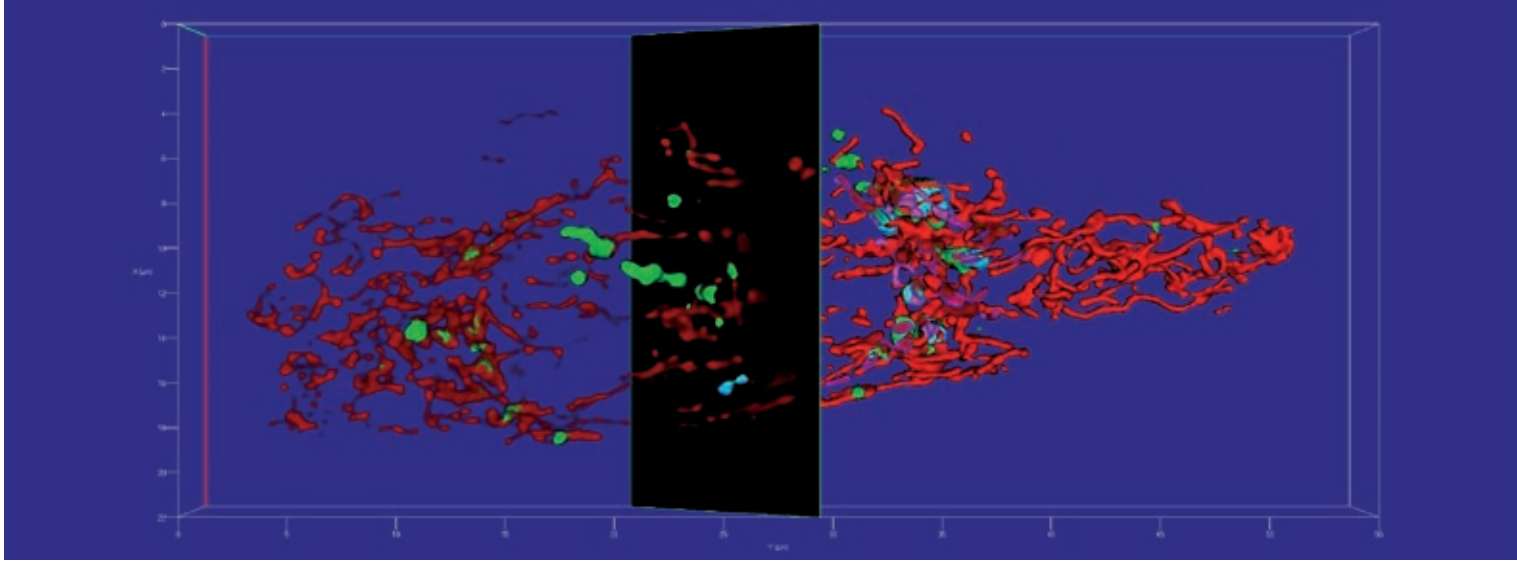
2) Diagram of colocalization analysis showing the colocalization coefficients of Pearsons and Manders. Whereas Manders' colocalization provides purely qualitative information, Pearsons' also shows the significance of the concentrations of the components concerned.

Combinatorics: hippocampal neurons in overview and in detail

Cell Observer® is so flexible that even entirely different applications can be freely combined. The images show embryonic hippocampus cultures – an ideal preparation for performing neurobiological cell studies of all kinds. Primary CD1 mouse hippocampal neurons were transfected with the two presynaptic marker proteins synaptotagmin-GFP and synaptophysin-mCherry. Axonal transport was monitored over 400 time points in two channels and two z-planes over a period of three minutes (insert within large image, scale bar = 2 µm). Axonal vesicle transport can also be tracked in the static display (arrow). In another experiment (large image) neurons of the same type were transfected with mRFP and PSD95-GFP. mRFP stains the entire cell body of the neuron, whilst PSD95-GFP (postsynaptic density protein) stains postsynaptic vesicles (green), as they fuse with surrounding cells. The cell nuclei are stained using DAPI. The cells were fixed and acquired as a 3-channel MosaiX image in 3 x 3 tiles (approx. 4,000 x 3,000 pixels) using the 63x Plan-APOCHROMAT 1.4 objective. To increase the image resolution, ApoTome was employed during acquisition. Although entirely different in terms of their technical requirements, both applications can be performed within a few minutes and without major effort using Cell Observer®.

Quantification with Colocalization

Often fluorescence microscopy is the only way to analyze the structural relationship of selectively stained structures. In addition to an overlaid color display, which is often open to subjective interpretation, the Colocalization module offers reliable quantification of colocalization, e.g. a display of two channels in the form of a scatterplot. All standard measurements, such as Pearsons' and Manders' Correlation Coefficients, are displayed (see diagram).



HeLa cell transfected with reggie-1/flotillin-2-eGFP (green). Mitochondrial staining with MitoTracker Red. Time lapse image of 21 z-planes in two channels, interval between time points 3.5 seconds. Z-stack was deconvolved using measured PSFs and rendered using Inside4D. The figure shows a time point with a mixture of isosurface and volume rendering. It is possible to see the tubular mitochondria and large and small reggie vesicles.
Image from the laboratory of Prof. Claudia Stürmer, University of Konstanz, Germany

3D Deconvolution for very dim structures: reggie vesicles in HeLa cells

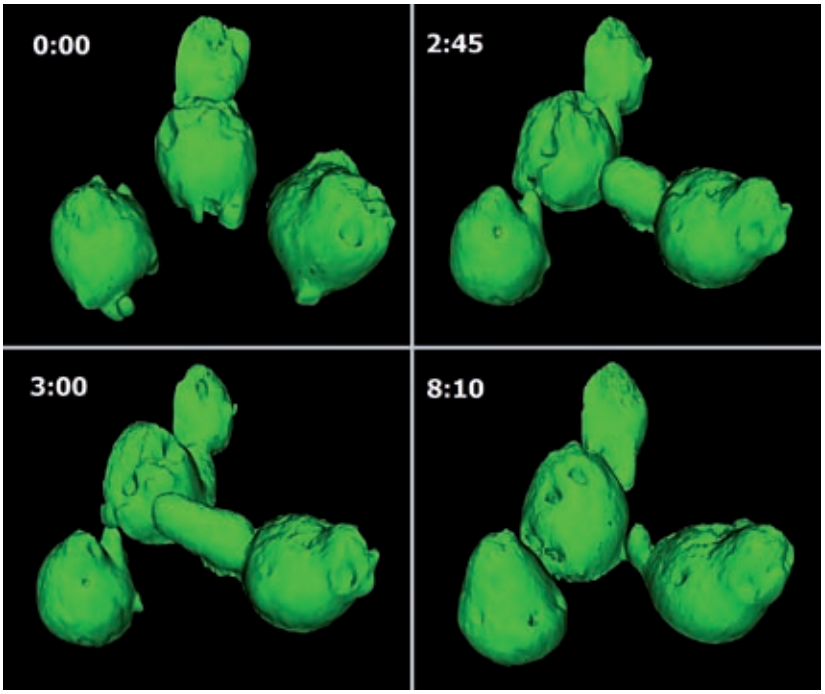
To acquire images of rapidly moving intracellular processes such as vesicle transport or the movement of mitochondria you need an acquisition technique that is both rapid and gentle to the sample. Minimizing phototoxicity and achieving speed during the acquisition of the Z-stack in order to freeze the movement are essential for successful Live Cell Imaging.

Making use of knowledge of the point spread function (PSF), 3D Deconvolution calculates light back to its place of origin. Elements that are not in focus are eliminated. This increases contrast and even the dimmest of structures are made visible. The huge advantage for the user: close integration between acquisition and processing.

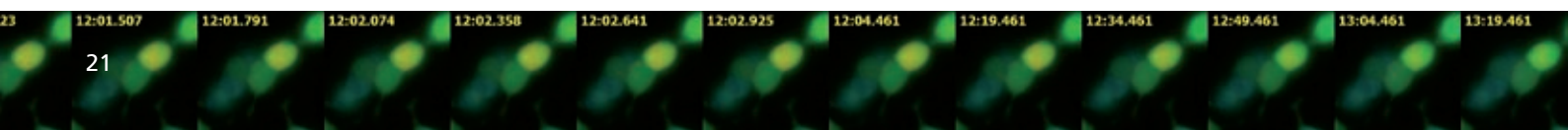
The movement of dictyostelium in 45 z-planes

Dictyostelium is an important model system in developmental biology. In order to secure their survival, these single-cell organisms pass through multi-cellular stages of development.

Images of these cells, which move rapidly in the single-cell stage, can be acquired three-dimensionally with Cell Observer® HS. In the experiment 45 z-planes were acquired in 2.5 seconds, and an image stack every 5 seconds, with 100 time points being acquired in approximately 8 minutes. Post-processing using 3D Deconvolution and 4D rendering shows four cells that have clearly retained their viability.



Four dictyostelium cells shortly after spore germination in the vegetative stage, stably transfected with a GFP construct. The pseudopodia growth that is typical for this stage is clearly visible.
Cells: Dr. Ralph Gräf, University of Potsdam, Germany

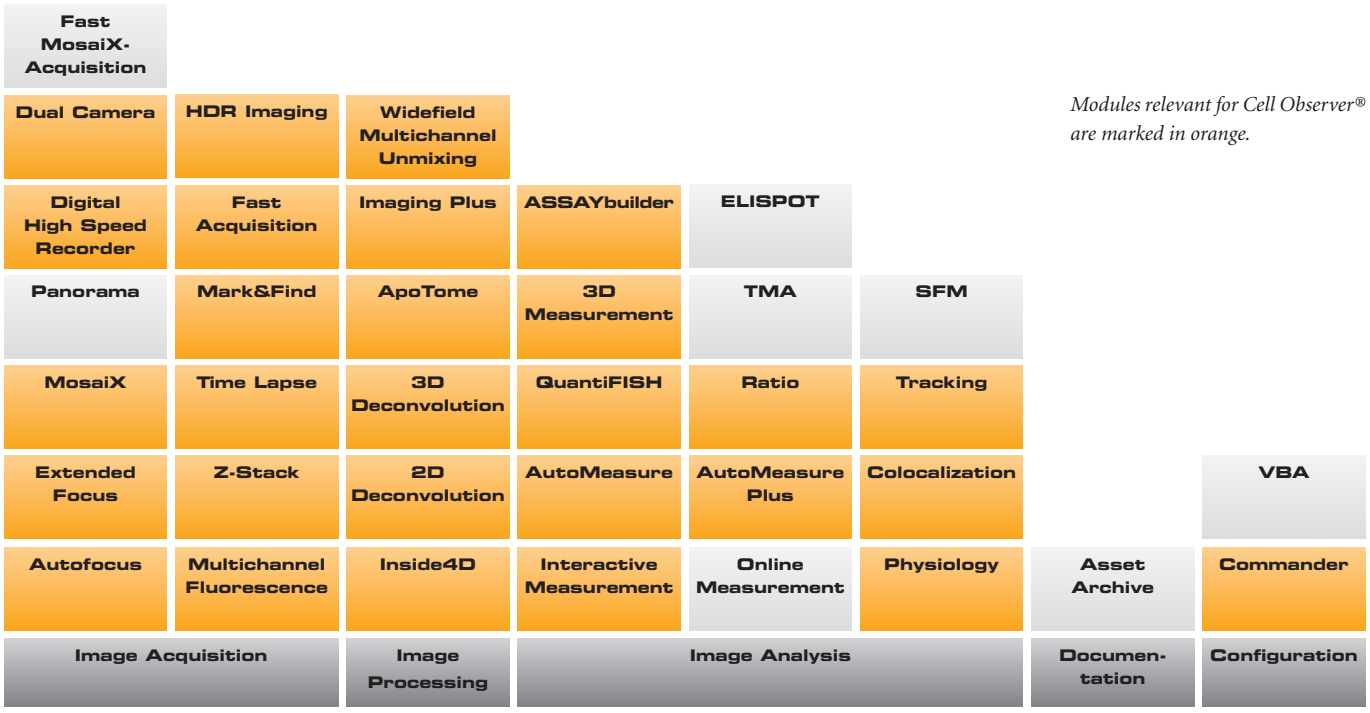


Future-oriented Software Grows with Your Tasks.

AxioVision is the intelligent software platform for Digital Imaging in the field of microscopy. The basic package can be expanded to open up a range of highly sophisticated applications, meaning that AxioVision grows economically with your tasks.

Image acquisition modules

- **Multichannel Fluorescence:** acquisition of up to 32 channels in fluorescence and various transmitted light techniques such as phase contrast or Nomarski (DIC).
- **Time Lapse:** generation of image series over time, which can be played back as films and processed further. Contains Smart Experiment function for acquisition of heterogeneous experiments.
- **Z-Stack:** three-dimensional images by means of image sequences from different focus planes.
- **Mark&Find:** acquisition of different positions of a specimen, e.g. from multiwell plates.
- **MosaiX:** tile-by-tile acquisition of large specimens at high magnification. Tiles can be merged to produce a very large high resolution composite image.
- **Autofocus:** automatic refocusing during time lapse and multichannel image acquisition
- **Fast Acquisition:** activates fast-acquisition functions for the Multichannel Fluorescence, Time Lapse and Z-Stack modules. Basic module for Cell Observer® HS (see Technical Info).
- **Physiology:** acquisition of even extremely rapid changes in ion concentrations. Powerful and intuitive online analysis functionality during acquisition.
- **ApoTome:** together with the ApoTome slider you obtain high-resolution optical sections of your samples



Modules relevant for Cell Observer® are marked in orange.

- **Dual Camera:** simultaneous acquisition from two identical cameras, e.g. for FRET or Emission Ratio Imaging.

Processing modules

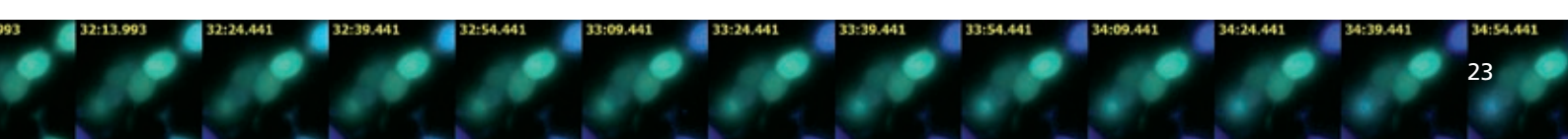
- **Deconvolution:** basis for 3D microscopy with widefield fluorescence images. Light outside the focus plane is calculated back to its place of origin. The result: higher contrast, better resolution and the recycling of valuable signals. 2D Deconvolution as an entry-level version.
- **Inside4D:** high-performance and highly functional rendering module with five different display methods, perfectly integrated into the workflow of acquisition ⟶ processing ⟶ display.
- **Widefield Multichannel Unmixing:** for multichannel images with crosstalk. Crosstalk is unmixed quantitatively, resulting in substantial signal enhancement and better light detection efficiency at the same time.

Image analysis modules

- **ASSAYbuilder:** fully automatic high content analysis of multichannel images. Cells are identified as objects. Analysis of both morphological and intensity-based parameters from all channels.
- **AutoMeasure:** combines the most important image analysis functions in an easy-to-operate software wizard. Generation of sophisticated measurement programs and automatic application to an entire image series.
- **AutoMeasure Plus/Imaging Plus/Commander:** possibility of freely combining a range of image processing and measurement functions. Processing of multidimensional or very large images. With Commander individual steps can be combined to create comprehensive scripts.
- **Interactive Measurement:** interactive measurement of images on the basis of geometric and densitometric parameters.
- **Colocalization:** essential when it comes to the quantitative analysis of multichannel images. Generation of reliable data on the relationships between structures in the cell.
- **Physiology:** function for analyzing time lapse images. Measurement of changes in intensity over time. Ratiometric measurement of changes in ion (e.g. calcium) concentrations.
- **3D Measurement:** volume analysis of 3D data sets.
- **Tracking:** interactive and semi-automatic tracking of cells and intracellular structures.

Differentiation Matrix Between Cell Observer® and Cell Observer® HS

Modul name	Multidimensional acquisition	Fast multidimensional acquisition
Multichannel Fluorescence	YES (up to 32 channels)	YES (up to 5 channels)
Time Lapse	YES	YES
Z-Stack	YES	YES
MosaiX	YES	NO
Mark&Find	YES	NO
Smart Experiments	YES (included in Time Lapse module)	NO
Fast Acquisition	NO	YES (module is a prerequisite – activates fast acquisition functions of the Multichannel Fluorescence, Time Lapse and Z-Stack modules)
Physiology	YES (analysis after acquisition only)	YES
Dual Camera	NO	YES (prerequisite for FRET and Emission Ratio Imaging with Physiology)

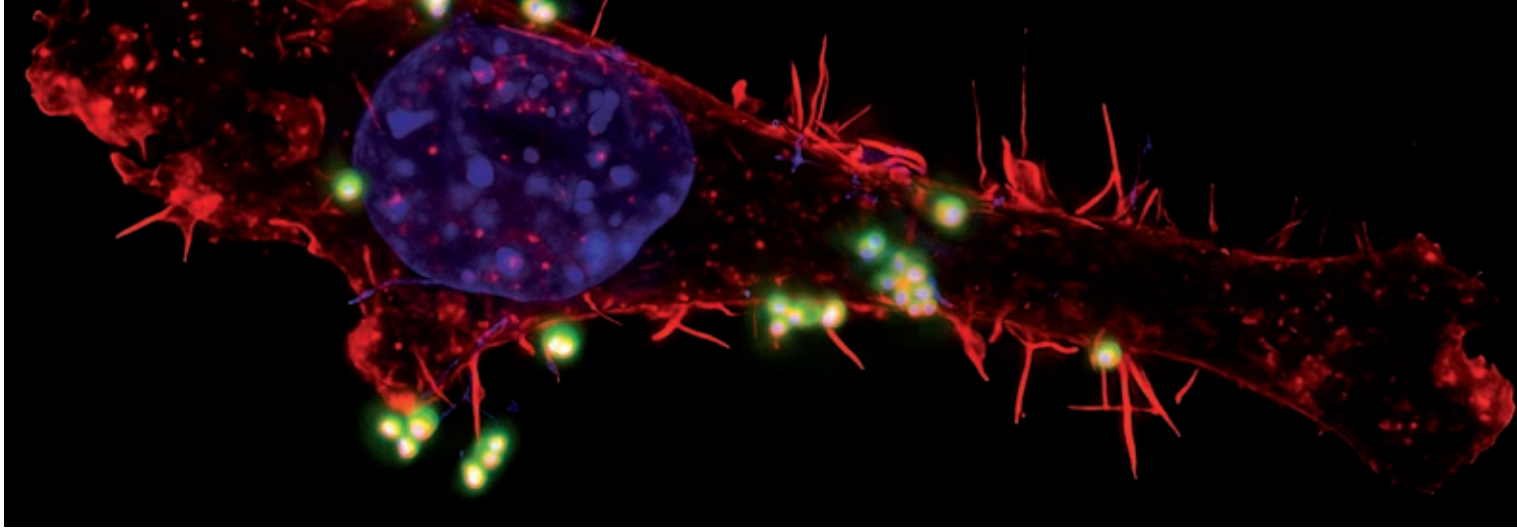


Recommended Configuration for Cell Observer® HS with Colibri

Areas of application (examples):

- Multidimensional High Speed Imaging of cell cultures
- Combination of Multichannel, Time Lapse and Z-Stack with Fast Acquisition
- Gentle High Speed 3D Imaging in conjunction with mature deconvolution algorithms
- Imaging of most of the available fluorescent proteins (BFP, CFP, GFP, YFP, DsRed, mRFP, HcRed, mCherry etc.)
- Physiology: Calcium and ion measurements (with Fura-2 use of the alternative light source Lambda DG-4 is recommended)
- MosaiX: Multidimensional acquisition of larger specimen (using standard multidimensional acquisition)

Group	Component	Order number	Comment
Microscope	Axio Observer.Z1	431007-9901-000	For additional equipment required, e.g. condenser or reflector revolver etc., see Axio Observer brochure
Objectives	C-APOCHROMAT 40x/1.2 W. Korr.	421767-9970-000	Top-class water-immersion objective for specimens in aqueous medium
	LCI Plan-NEOFLUAR 63x/1.3 Imm. Korr. Ph3 M27	420881-9970-000	Phase contrast objective specially designed for the requirements of live cell microscopy, water or glycerol immersion
	Plan-APOCHROMAT 63x/1.40 Oil DIC	420782-9900-000	Oil objective with extremely high resolution
Filter sets	59 HE CFP + YFP shift free	489059-0000-000	High-efficiency multi-band pass filter set for Colibri
	62 HE BFP + GFP + HcRed shift free	489062-0000-000	High-efficiency multi-band pass filter set for Colibri
Incubation	Incubator XL S1	411857-9060-000	Incubator for optimum thermal stability of overall configuration
	Heating Insert P S1; compact	411861-9902-000	Heating insert specially designed for scanning stage with piezo top plate
	CO_2-Cover HP for Heating Insert P S1	000000-0441-341	Cover for CO_2 application/humidification
	Heating Unit XL S1	411857-9030-000	Heating unit for XL
	TempModule S1	411860-9010-000	Control module, basis for all control units, connected to PC
	CO_2 Module S1	411857-9010-000	CO_2 regulation
	Control Sensor T S1	411857-9080-000	Sensor for measuring temperature directly at the place of observation
Camera	AxioCam HRm Rev.3	426511-9910-000	Peltier-cooled, high-resolution monochrome CCD camera
	AxioCam HSm	426507-0000-000	High-speed model for acquisition of extremely rapid processes
Computer	2.0 GHz dual core XEON image analysis workstation incl. 3 hard drives	410203-9902-000	High-end workstation for maximum data throughput (e.g. 2 hard drives as RAID 0 array) and expandability; recommended equipment: open GL graphics card with 1 GB RAM, 3 GB RAM, second CPU, additional 750 GB hard drive etc.
	Trigger board for fast acquisition	410358-0100-000	Direct control of high-speed components



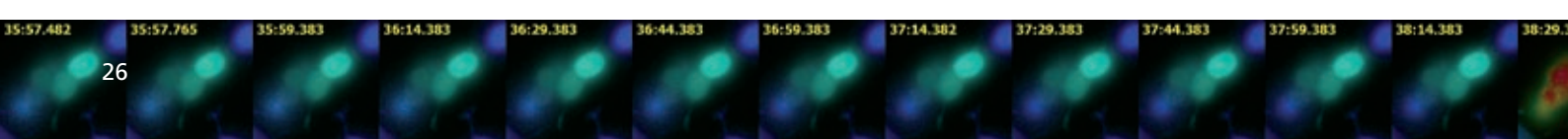
Group	Component	Order number	Comment
Light sources	Colibri LED illumination system	423052-9500-000	See Colibri brochure for further details
	LED module 365 nm	423052-9010-000	
	LED module 455 nm	423052-9040-000	
	LED module 470 nm	423052-9050-000	
	LED module 505 nm	423052-9060-000	
	LED module 590 nm	423052-9080-000	
	HXP 120 illumination equipment	423013-0000-000	Metal-halide lamp as additional white light source
Microscope stage	XY DC 100x100 scanning stage with piezo top plate	000000-0473-167	Scanning stage with piezo top plate focusing (100 µm traveling range)
Accessories	High-speed shutter, external	423627-0000-000	For use in the transmitted-light channel, combination of phase contrast and fluorescence
	Uniblitz shutter controller VCM-D1	000000-1343-597	Control unit for shutter
Software	AxioVision Basic Software	410130-0300-000	Acquisition modules and driver modules: Basic AxioVision software already includes microscope and camera control. Driver modules are required for certain components from third-party manufacturers.
	AxioVision 4 Multichannel Fluorescence, Z-Stack, Time Lapse modules	000000-1222-047	
	AxioVision 4 Fast Acquisition module	410131-0200-000	
	AxioVision 4 Physiology module	410132-0306-000	
	AxioVision 4 Autofocus module	000000-1235-856	
	AxioVision 4 MosaiX module	000000-1235-913	
	AxioVision 4 Mark&Find 2 module	000000-1304-587	
	AxioVision 4 software driver for DC scanning stage with z-piezo attachment	410131-0200-011	
	AxioVision 4 software driver for Uniblitz shutter controller VCM-D1/VMM-D1	000000-1222-050	
	AxioVision 4 3D Deconvolution module	000000-1235-922	Recommended processing and analysis modules
	AxioVision 4 Colocalization module	410131-0200-002	
	AxioVision 4 Commander module	000000-1235-863	
	AxioVision 4 Inside4D module	000000-1222-044	
	AxioVision 4 Interactive Measurement module	000000-1235-871	
	AxioVision 4 Widefield Multichannel Unmixing module	000000-1305-375	

Recommended Cell Observer® Configuration for Multiwell and Long-term Time Lapse Applications

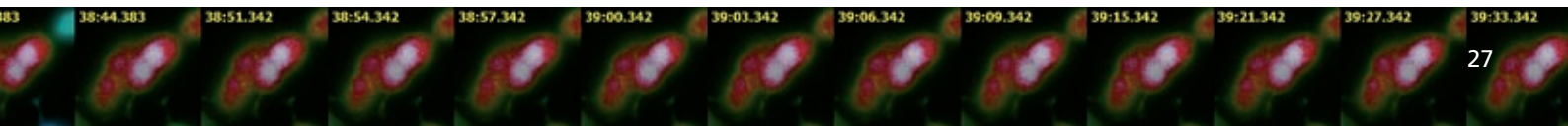
Areas of application (examples):

- Time lapse images over long periods (experiments lasting several days are possible, depending on equipment)
- Extensive options for combining Multidimensional Acquisition dimensions
- Imaging of most of the available fluorescent proteins (BFP, CFP, GFP, YFP, DsRed, mRFP, HcRed, mCherry, etc.)
- MosaiX: Multidimensional Acquisition of larger specimen, in particular in combination with ApoTome
- Screening applications: acquisition of samples on multiwell plates. Can be combined with high content analysis

Group	Component	Order number	Comment
Microscope	Axio Observer.Z1	431007-9901-000	For additional equipment required, e.g. condenser or reflector revolver etc., see Axio Observer brochure
Objectives	EC Plan-NEOFLUAR 5x/0.16 Ph1 M27	420331-9911-000	Overview objective suitable for fluorescence for AxioVision MosaiX and Mark&Find modules
	EC Plan-NEOFLUAR 10x/0.3 Ph1 M27	420341-9911-000	Ideal for screening of multiwell plates
	Plan-APOCHROMAT 20x/0.8 M27	420650-9901-000	High-resolution air objective, ideal for all screening applications with vessels featuring glass cover-slip bottoms
	Plan-APOCHROMAT 40x/1.3 Oil	420762-9800-000	High-aperture oil immersion objective, excellent compromise between large field of view and high resolution
	LCI Plan-NEOFLUAR 63x/1.3 Imm. Korr. Ph3	420881-9970-000	For detailed images and Z-stacks, multi-immersion – specially designed for live cell microscopy
Filter sets	49 DAPI shift free	488049-0000-000	Standard DAPI and Hoechst filter
	38 HE eGFP shift free	489038-0000-000	Wide range of uses for blue-excitable dyes such as GFP and FITC; high-efficiency technology
	43 HE Cy 3 shift free	489043-0000-000	Wide range of uses for green-excitable dyes such as Cy3 or Texas Red; high-efficiency technology
	50 Cy 5 shift free	488050-0000-000	Deep-red excitation
	59 HE CFP + YFP shift free	489059-0000-000	High-efficiency multi-band pass filter set for Colibri
	62 HE BFP + GFP + HcRed shift free	489062-0000-000	High-efficiency multi-band pass filter set for Colibri
Incubation	Incubator XL S1	411857-9060-000	Incubator for optimum thermal stability of overall configuration
	Heating Insert P Lab-Tek™ S1	411860-9025-000	Heating insert for Lab-Tek™ chamber slides and Petri dishes
	CO_2-Cover PM S1	411857-9110-000	Cover for CO_2 application/humidification
	Universal Mounting Frame K-M	000000-1272-644	Can be used for all types of multiwell plates; here heating takes place solely via Incubator XL S1
	CO_2-Cover HM	000000-0441-342	CO_2 application and humidification cover for Universal Mounting Frame K-M
	Heating Unit XL S1	411857-9030-000	Heating unit for XL
	TempModule S1	411860-9010-000	Control module, basis for all control units, connected to PC
	CO_2 Module S1	411857-9010-000	CO_2 regulation



Group	Component	Order number	Comment
Incubation	Control Sensor T S1	411857-9080-000	Sensor for measuring temperature directly at the place of observation
Camera	AxioCam HRm Rev.3	426511-9910-000	Peltier-cooled, high-resolution monochrome CCD camera
Computer	2.0 GHz dual core XEON image analysis workstation incl. 3 hard drives	410203-9902-000	High-end workstation for maximum data throughput (e.g. 2 hard drives as RAID 0 array) and expandability; recommended equipment: open GL graphics card with 1 GB RAM, 3 GB RAM, second CPU, additional 750 GB hard drive etc.
Light sources	Colibri LED illumination system	423052-9500-000	LED modules for 365 nm, 455 nm, 470 nm, 505 nm, 590 nm, 626 nm are available. See Colibri brochure for further details of equipment
	HXP 120 illumination equipment	423013-0000-000	Metal-halide lamp as additional universal white light source
Microscope stage	120x100 STEP scanning stage	432029-0000-000	Scanning stage with stepper motors, traveling range is multiwell compatible, adjustable traveling speed
	Ludl MAC5000 XY stage controller stepper including joystick	000000-0431-478	Required in combination with Incubator XL: cable set 000000-0445-551
ApoTome	ApoTome slider system	000000-1189-776	Insert for generating optical sections, ideal when working with thicker specimens
Software – Acquisition	AxioVision core	410130-0300-000	Acquisition modules and driver modules: Basic AxioVision software already includes microscope and AxioCam control. Driver modules are required for certain components
	AxioVision 4 Multichannel Fluorescence, Z-Stack, Time Lapse modules	000000-1222-047	
	AxioVision 4 Mark&Find 2 module	000000-1304-587	
	AxioVision 4 ApoTome module	000000-1222-053	
	Software driver for Autofocus	000000-1235-856	
	AxioVision 4 Modul MosaiX	000000-1235-913	
	Software driver for LUDL	000000-1235-874	
Software – Analysis and processing	AxioVision 4 3D Deconvolution module	000000-1235-922	Recommended processing and analysis modules
	AxioVision 4 Colocalization module	410131-0200-002	
	AxioVision 4 Inside4D module	000000-1222-044	
	AxioVision 4 Widefield Multichannel Unmixing module	000000-1305-375	
	AxioVision 4 AutoMeasure Plus module	000000-1281-793	
	AxioVision 4 Imaging Plus module	000000-1235-869	
	AxioVision 4 Commander module	000000-1235-863	
	AxioVision 4 Interaktive Measurement module	000000-1235-871	
	AxioVision 4 ASSAYbuilder Core	410132-0311-000	
	AxioVision 4 module Physiology Analyst for ASSAYbuilder	410132-0312-000	
	AxioVision 4 module Membrane Analyst for ASSAYbuilder	410132-0313-000	
	AxioVision 4 module Motility Analyst for ASSAYbuilder	410132-0314-000	
	AxioVision 4 module Cell Cycle Analyst for ASSAYbuilder	410132-0315-000	
	AxioVision 4 module Morphology Analyst for ASSAYbuilder	410132-0316-000	



Cell Observer® from Carl Zeiss

Target groups and areas of application

- Cell biology
  Ciliary beat analysis
  Cell division
  Cell structure analysis: actin, cytoskeleton, cell nucleus
  Intracellular transport dynamics: Golgi/ER, nucleo/cytoplasmic transport
- Neurobiology
  Ion concentration
  Cell differentiation
- Zoology
  Parasitology
  Protozoology
  Reproduction
- Limnology
  Development and behavior of water-borne organisms
- Physiology
  Ion concentration measurements (Calcium Imaging)
- Botany
  Cell morphology and development in plants
  Phytoplankton Research
- Developmental biology
  Cell division and tracking
  Gene expression analysis
  Mutation analysis
- Pharmacology
  Research into active ingredients with the help of cell cultures

Functions

- Acquisition in allcommon contrast techniques (brightfield, darkfield, fluorescence, phase contrast, DIC, PlasDIC)
- Any combination of different dimensions with Multidimensional Acquisition: time lapse series, Z-stacks, multichannel, mosaic images, multiposition imaging, optical sectioning
- Long-term observation
- High-speed mode for acquisition of extremely rapid processes
- Heterogeneous time lapse experiments
- Scanning of multiwell plates and arrays for high-throughput microscopy
- Hardware can be controlled entirely via the software
- Flexible selection of components, adapted to the application
- Targeted adjustment of environmental parameters during an experiment

Carl Zeiss Microscopy GmbH
07745 Jena, Germany
BioSciences
microscopy@zeiss.com
www.zeiss.de/cellobserver

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