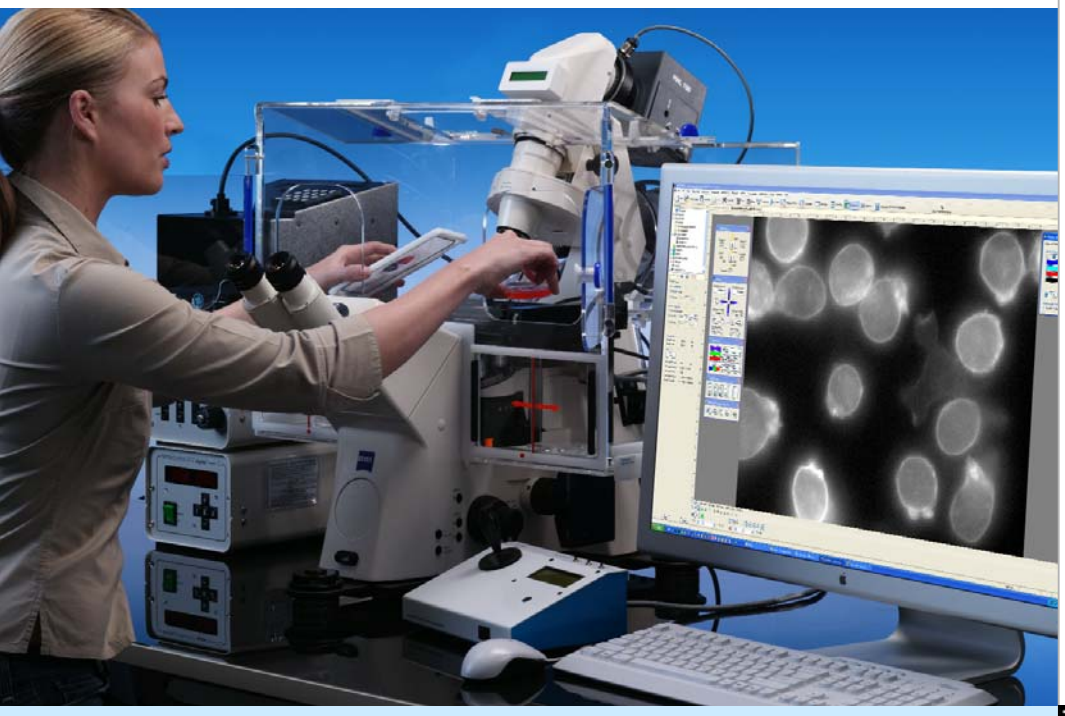
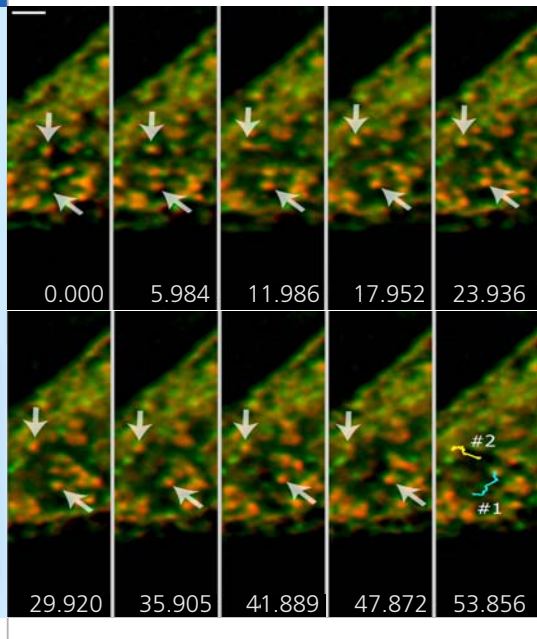
Cell Observer® HS

Speed has Many Dimensions. The Crucial One is Performance.



Whether you are working in the areas of cell, developmental or neurobiology, zoology or virology – modern digital microscopy applications in the field of life science research are focusing increasingly on the quantitative observation of rapid processes. Today, a wealth of fluorescence markers are available for this purpose. In order to capture the speed of such highly dynamic processes, you need acquisition technology that is powerful, ultra-fast and easy to operate.

Figure 1: 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. Measurement of two particles in DsRed channel with the Tracking module (see table for analysis). Houchaima Ben Tekaya, Biozentrum, University of Basel, Switzerland, scale bar: 2 µm.
www.zeiss.de/cellobserver-gallery



The Cell Observer® from Carl Zeiss has long been an established system solution for live cell imaging. With the Cell Observer® HS (High Speed), Carl Zeiss is now introducing the next generation into the market. Crucial advances have been made allowing all elements to be controlled directly via the hardware. Each of the perfectly coordinated components – from the microscope, camera, fast-switching light source and shutter through to the focusing equipment – have been optimized for maximum speed.

Track #	Start Frame	End Frame	Time [sec]	Total Distance [µm]	Straight 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

The New Cell Observer® Generation for Live Cell Imaging of the Most Rapid of Processes as well as for Observation Over Extended Periods of Time.

ZEISS

We make it visible.

When You Carry Out Research in Life, Every Millisecond Counts. And Sometimes Every One of Your 2,700 Images.

Dynamic processes unfolding extremely quickly in living cells demand rapid time lapse image acquisition procedures that are able to match this speed – which means that the simultaneous exposure and readout of the sensor in the camera is required. The AxioCam microscope camera with its streaming technology and the optimized synchronization of all external components of the Cell Observer® HS enable image rates of up to 200 images per second to be achieved.

High-end technology for the analysis of ciliary beat frequency

One possible application is the analysis of the ciliary beat of lung epithelial cells, as illustrated in figure 2 (samples: Dr. Bob Hard, University of Buffalo, USA), in Differential Interference Contrast (Nomarski). The cilia of these amphibian cells beat approximately 30 times per second depending on the temperature. To allow precise analysis, an acquisition frequency of 153 images per second with an exposure time of 2 ms is required. For quantitative analysis, subsequent images are subtracted from each other in order to extract information about changes in the image sequence. Displaying the information of the 2nd order derivation means that only accelerating components in the image are made visible. This information is pseudocolored in red in the merged image.

Maximum frame rates with streaming technology

Even the most rapid processes can be acquired with precision with the AxioCam HSm. The sensor, with 660 x 494 pixels, is read out at 24.57 MHz. In addition, the use of streaming technology makes simultaneous exposure and readout possible for rapid time lapse images in a single channel – and, what is more, at perfectly equidistant intervals. The highly sensitive AxioCam MRm can be used universally. It acquires even very weak fluorescence signals with up to 80 images per second, and streaming technology can be used for this camera too. All external components are fully integrated into the system and are synchronized with each other directly and precisely by means of trigger signals. This makes it possible to avoid image blur caused by movement and the loss of frames that are important to the experiment.

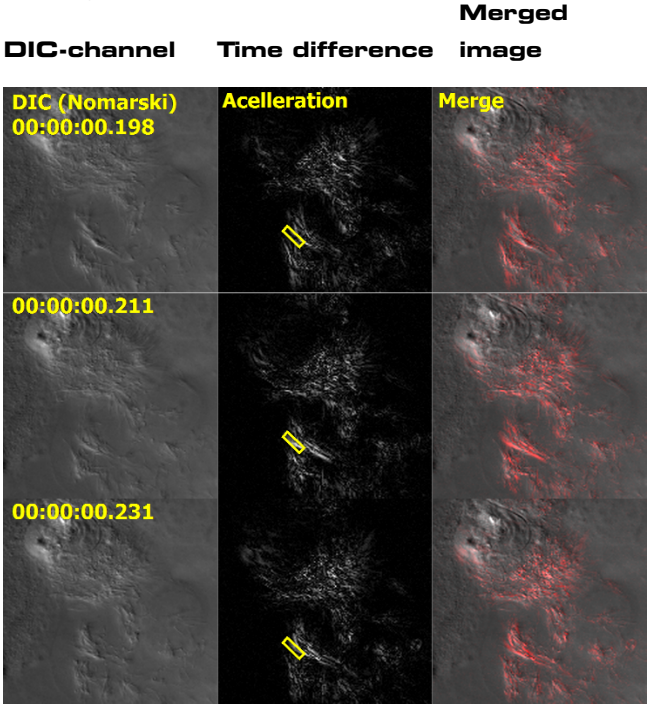
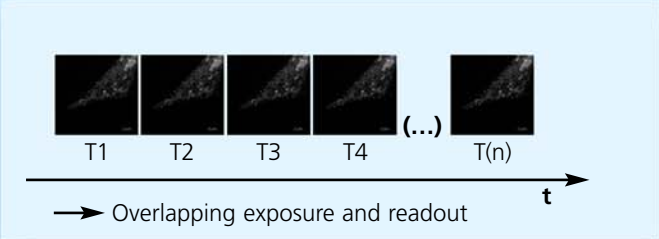
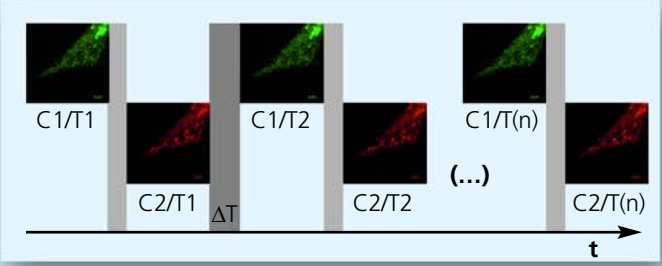


Fig. 2: Ciliary beat in newt lung epithelial cells. Merged image, channel 2 pseudocolored red. Measurement region: yellow rectangle.
Dr. Bob Hard, University of Buffalo, USA. ***www.zeiss.de/cellobserver-gallery***

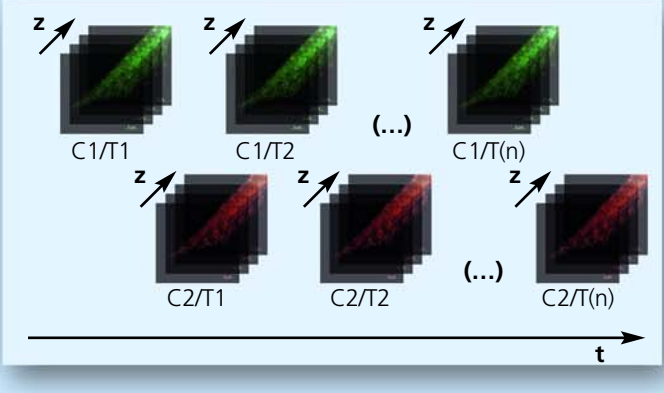
Frame rates for extremely high acquisition speeds



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

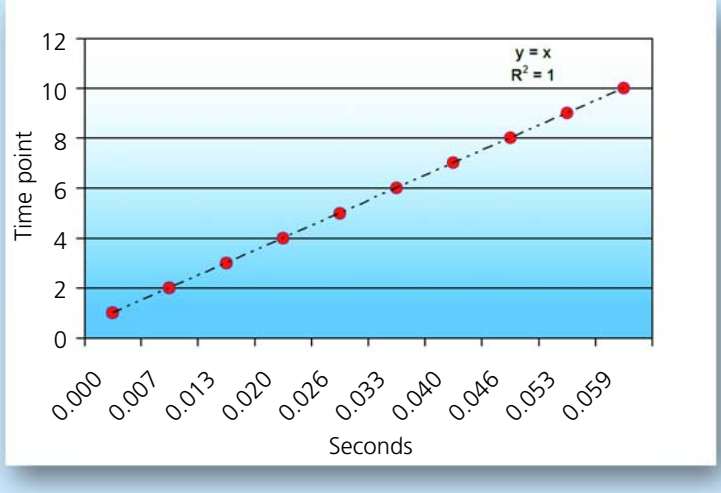


Dimensions			Frames/second	
Channels	z-planes	Binning/ROI	AxioCam HSm	AxioCam MRm
2	1	no	31	7
		yes	94	29

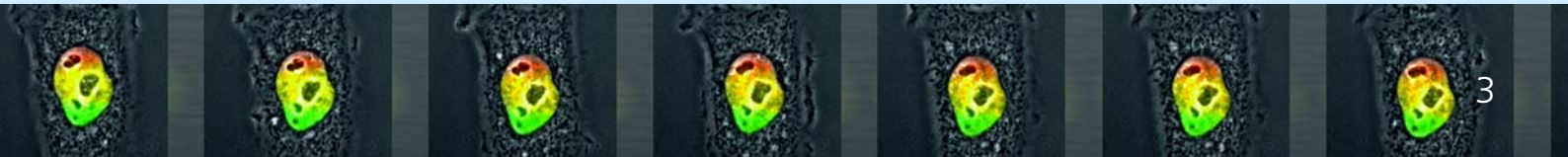


Dimensions			Frames/second	
Channels	z-planes	Binning/ROI	AxioCam HSm	AxioCam MRm
2	20	no	24 (= 1.7 sec/ instant of exposure)	7 (= 5.6 sec/ instant of exposure)
		yes	40 (= 1.0 sec/ instant of exposure)	25 (= 1.6 sec/ instant of exposure)

Equidistant time lapse images at 153 images per second



The chart shows the relative acquisition time against the respective time point. Deviations in the intervals between the individual time points would result in an R2 value < 1 (regression line). The R2 value of the line shown is 1. Ten time points are shown derived from a time lapse series containing 200 images.



To Make the Most Rapid Processes Visible, Each Component Has to Meet One Particular Criterion: Perfection.

If multichannel fluorescence signals with different markers are to be documented simultaneously, rapid switching of the excitation wavelengths is required. The Cell Observer® HS offers two alternatives for rapid Xenon light sources and newly developed multiple bandpass filter sets. This guarantees optimum excitation of the fluorochromes and protects the sensitive samples.

Precise Z-stack images of living trypanosomes in two channels

Living trypanosomes move at a very high speed with the help of an undulating membrane. Figure 3 shows images acquired in two channels (Mitrotracker Red and DAPI) and 30 z-planes per time point. Each time point is acquired in 2.7 seconds. The image series is made up of 20 image stacks with a total of 1,200 individual images.

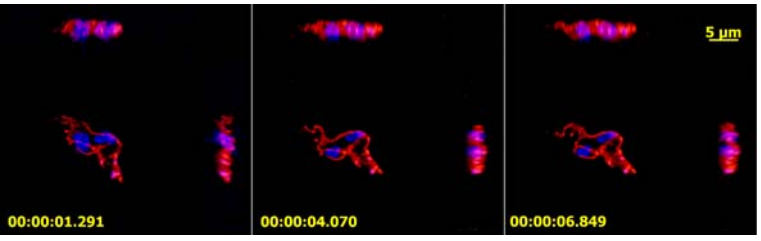


Fig. 3: Living trypanosomes.
Dr. Torsten Ochsenreiter, Woods Hole, Massachusetts, USA.
www.zeiss.de/cellobserver-gallery

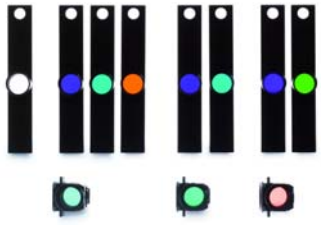
Two light sources for a quick change of wavelength

Bright illumination of the entire field of view, a long life span and, in particular, the same intensity over virtually the whole spectral range make the Sutter Lambda DG-4 Xenon lamp the ideal light source for all fluorescence markers in the visible range. It is possible to configure up to four independent filter positions and to switch between them in approximately 1.7-3 ms. In addition, the Sutter Lambda DG-4 features a light attenuation and shutter function.

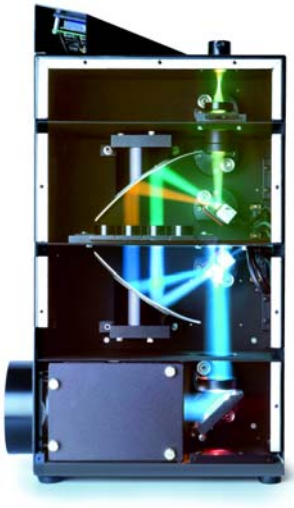
When highest UV illumination intensity is needed, with FURA-2 for example, the Till Polychrome V monochrome Xenon light source with a power of 150 Watts is ideal. It is coupled into the aperture diaphragm plane of the Axiovert 200 using a slider and a quartz fiber. The Till Polychrome V stands out due to its lamp durability, its homogeneous burning behavior and also due to the fact that it is possible to switch between wavelengths extremely quickly – in 1-3 ms.

The perfect addition: new multi bandpass filter sets

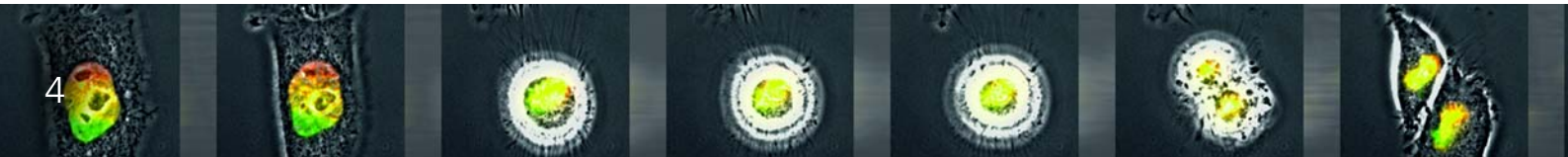
Both the Axiovert 200 and Axio Imager can be equipped with four new multi bandpass beam-splitter and emission filters. All filter sets feature high-efficiency technology: they offer maximum transmission, long life spans and extremely steep slopes. Up to five channels per experiment can be configured in combination with the Xenon light sources.



Combination of separate excitation filters and highly efficient multi bandpass beam-splitter and emission filters in standard Push&Click reflector cube: filter sets 54 (CFP, YFP, HcRed), 55 (CFP, YFP) and 56 (GFP as well as most variants of the red fluorescent proteins, such as DsRed).



Rapid switching of the light path simply through the movement of two small mirrors. Here two of the four possible light path settings are overlaid (generates green and red excitation light).



The Whole is as Good as Each of its Individual Components. And Just as Fast.

Piezo-based focusing devices are crucial for the documentation of rapid processes in 3D. These devices have to be able to reach the desired position not only with maximum focusing precision but also with maximum speed. The result: precise, three-dimensional images of rapidly moving objects in multiple z-planes.

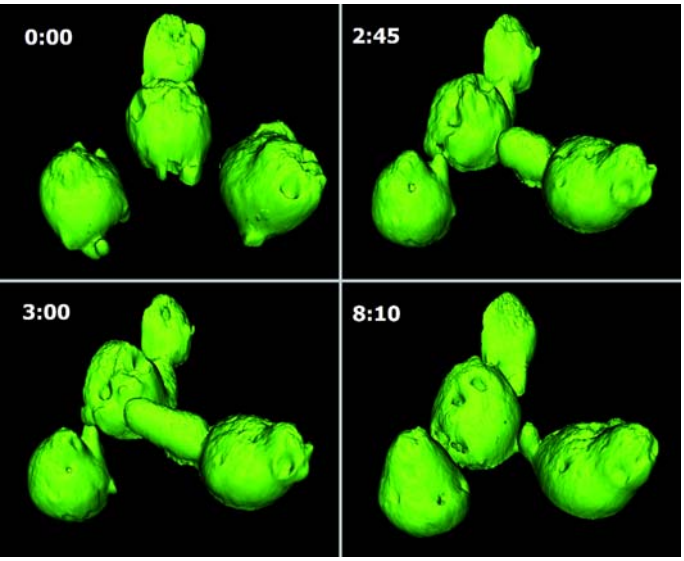


Fig. 4: Four dictyostelium cells shortly after spore germination in the vegetative stage, stably transfected with a GFP construct. The pseudopodia growth that is typical for this stage is clearly visible.
Dr. Ralph Gräf, Ludwig-Maximilians-University, Munich, Germany.
www.zeiss.de/cellobserver-gallery

The movement of dictyostelia in 45 z-planes

Dictyostelia are 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 fast-moving cells can be acquired three-dimensionally with the Cell Observer® HS. Figure 4 shows four cells following processing using 3D Deconvolution and 4D Rendering. It is clear that these cells have retained their viability.

Rapid focusing with the Piezo stage and Piezo objective unit

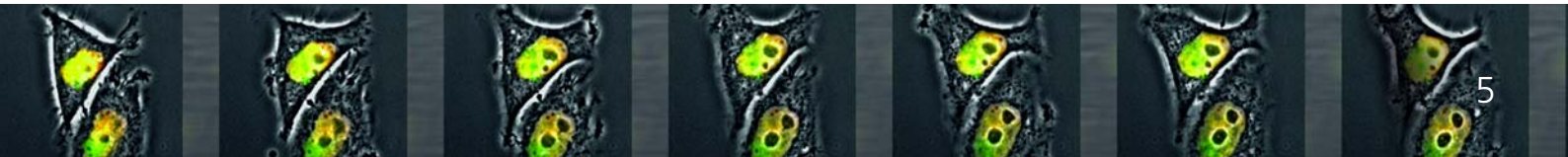
The Cell Observer® HS can be fitted with a Piezo stage insert with motorized x and y axes. The z-traveling range amounts to 100 µm in 1.5 nm steps. Up to 30 focus steps can be located in the z-direction per second. The Piezo stage is suitable for all objective positions and is compatible with all standard contrast procedures as well as incubation.

Two Piezo units that can move an objective in the z-direction with a traveling range of 100 or 250 µm are available for retrofitting to existing systems. Special height adapter rings are provided for the remaining objective positions. The Piezo objective unit can also be retrofitted to upright microscopes.

Intelligent coupling: the Uniblitz shutter

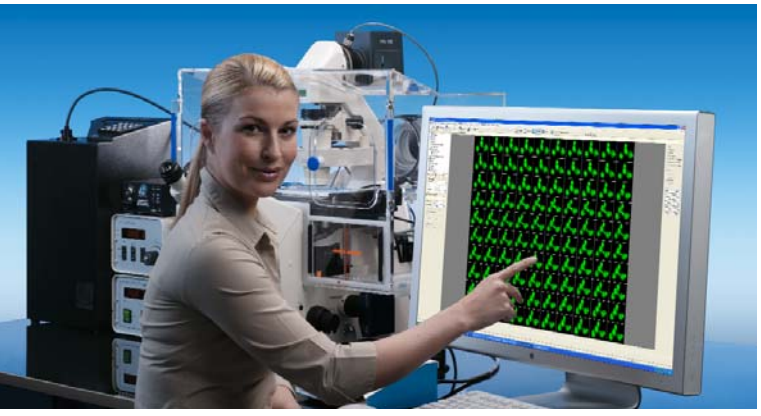
The tried-and-tested Uniblitz shutter is used for ultra-fast switching between the fluorescence and transmitted light beam path. It is inserted into the transmitted light beam path and has a switching time of 8 ms. Using this device, complex applications combining fluorescence with phase contrast or bright field can be carried out quickly and reliably.

Uniblitz shutter and control unit



Maximum Speed Requires Maximum Flexibility.

If you want to carry out research into very rapid processes in living cells, you cannot afford to allow weaknesses at any point. The Cell Observer® HS from Carl Zeiss is setting a new standard in live cell imaging – with outstanding fluorescence and unequaled Carl Zeiss optics, and without losing the variability and flexibility of image acquisition over long periods.



The microscopes: Axiovert 200 and Axio Imager

The Axiovert 200 inverse microscope and the Axio Imager upright microscope are both optimized for fluorescence and support the acquisition of rapid processes in live cell imaging particularly well. We recommend the motorized variants.

- Reliable and time-saving automation of many procedures
- Maximum stability
- Motorized reflector nosepiece with up to 10 filter positions (with Axio Imager.Z1)
- In combination with ApoTome for optical sections in the best resolution
- In combination with TIRF for rapid imaging of vesicle transport at the cell membrane

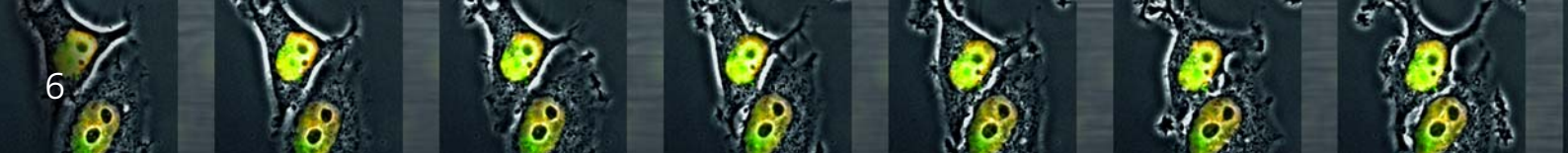
- Optimum fluorescence optics: high level of homogeneity of illumination, perfect contrast and brilliant imaging, even with weak light intensities
- Integration of all external components, such as shutters, light sources and focusing devices
- Perfect components for incubation of cells on the specimen stage

Status quo in optical design: the new LCI objectives

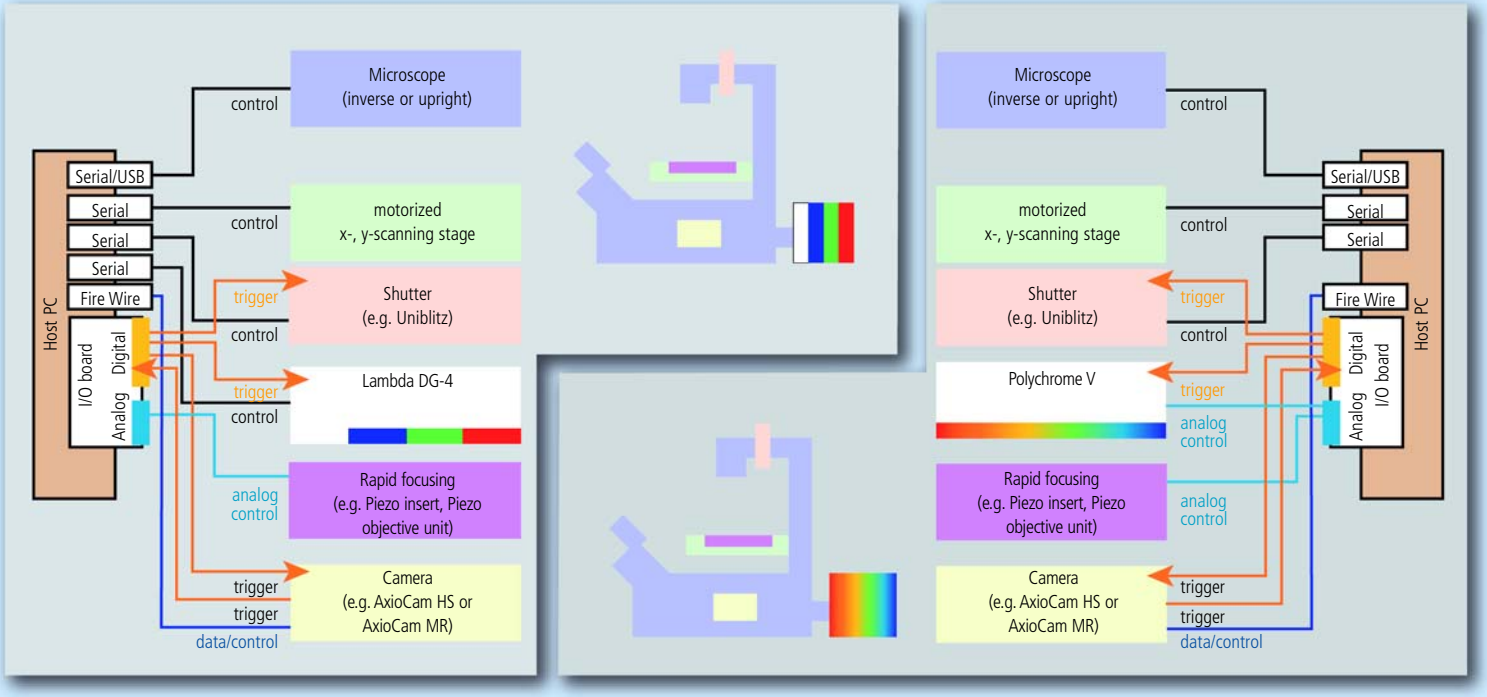
Carl Zeiss objectives, such as the Plan-Neofluar and Plan-Apochromat, have been developed for highly sophisticated applications and are recognized in the fields of science and research for their brilliant optics. The LCI Plan-Neofluar 63x/1.3 Imm Corr and LCI Plan-Neofluar 25x/0.8 Imm Corr have been newly designed especially for live cell imaging. They meet the highest demands in complex applications and stand out thanks to a range of significant features.

- Ideal for samples in liquid environments
- Excellent transmission properties
- Highest possible numerical aperture
- Correction ring to avoid spherical aberration: for 23° to 37°; immersion water, glycerol or oil (25x only)

Axio Imager and Axiovert 200



System diagram for Axiovert 200/Axio Imager with DG-4

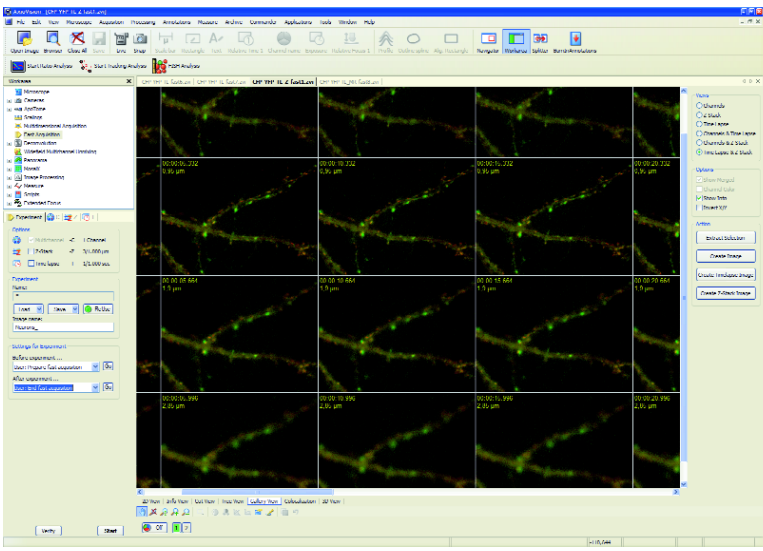


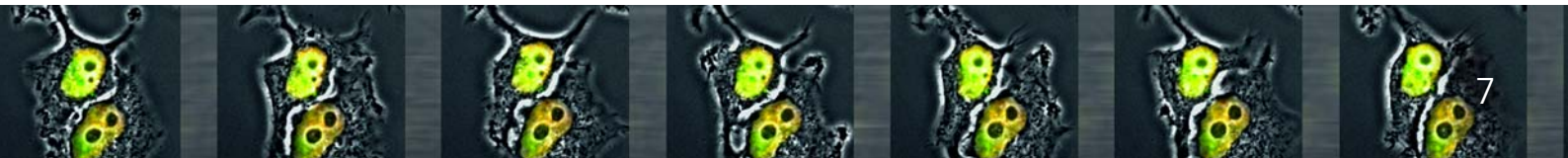
System diagram for Axiovert 200/Axio Imager with Polychrome V

Digital intelligence: AxioVision

The AxioVision software platform from Carl Zeiss is highly recognized in the fields of science and research. The Fast Acquisition module has been developed especially for the Cell Observer® HS. Every crucial detail of the software is perfectly geared towards fast image acquisition.

- New Fast Acquisition module
- Optimized support of new fast components
- Gallery view even for very large sequences
- Optimal analysis using a number of new modules, such as Colocalization, Tracking or Ratio
- Improved 3D Deconvolution module with new, rapid iterative algorithm
- New 2D Deconvolution module





Cell Observer® HS – Target groups and areas of application

Cell Biology	• Ciliary beat analysis • Cell division (cell cycle research) • Cell structure analysis: actin, cytoskeleton, cell nucleus, organelles • Intracellular transport dynamics: microtubules, nucleo/cytoplasmic shuttling
Neurobiology	• Ion concentration measurements (calcium ratio imaging) • Cell differentiation
Zoology **Limnology**	• Parasitology • Protozoology • Phytoplankton • Development and behavior of water-borne organisms
Molecular Cell Biology	• Molecular structure and function analysis in cell cultures • Intracellular transport of cellular components
Physiology	• Ion concentration measurements (calcium ratio imaging) • Electrophysiological phenomena
Botany	• Cell morphology and development in plants • Research into phytoplankton • Differentiation of root tip growth
Developmental Biology	• Early stages of development • Expression analysis of development-specific marker proteins

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