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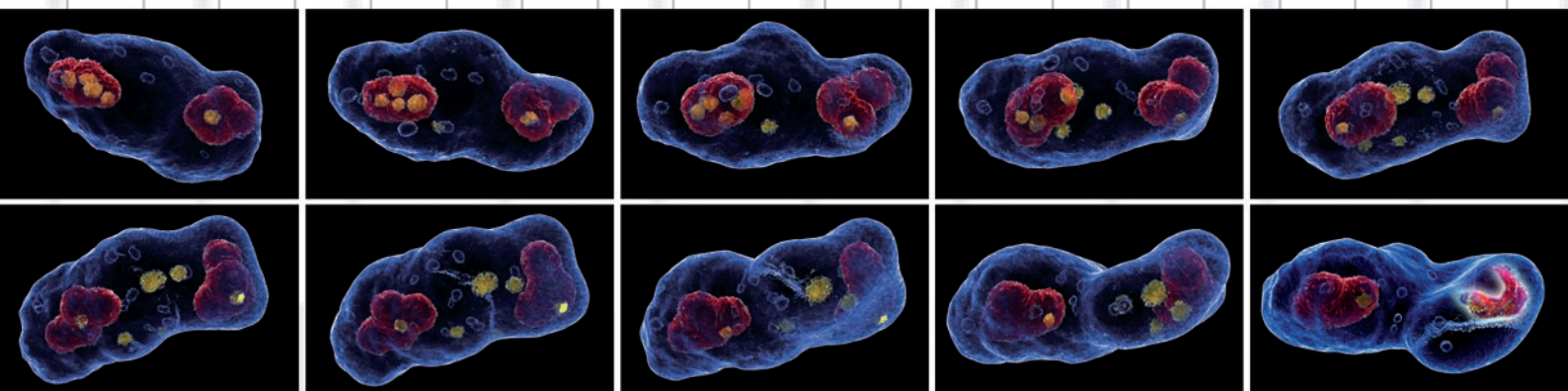
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# The Evolution of Digital Microscopy

## High-speed Image Acquisition with Cell Observer HS



### The Basis: Fully Integrated Cell Observer System

In 2001, Carl Zeiss launched Cell Observer, the first fully integrated live cell imaging system offered by a leading microscope manufacturer. This was the first system that combines a motorized inverted microscope (Axiovert 200M), a camera suitable for fluorescence work (AxioCam), diverse incubation devices and software for fully automatic control and image acquisition.

Rather than troubling the user with the laborious and time-consuming integration of components obtained from different manufacturers, the Cell Observer concept offers tailor-made, readily inte-

grated solutions that turn demanding multidimensional image acquisition tasks into routine.

The first-generation Cell Observer is mainly used for long term observation of cells expressing components labelled with fluorescent proteins at medium to slow speeds, and offers many functionalities for quantitative analysis. Some examples of such applications can be found under: [www.zeiss.de/cellobsever](http://www.zeiss.de/cellobsever) → Gallery

### The New Challenge: Speed

The last few years have seen an abundance of improvements for fluorescence-based applications with live cells. Mean-

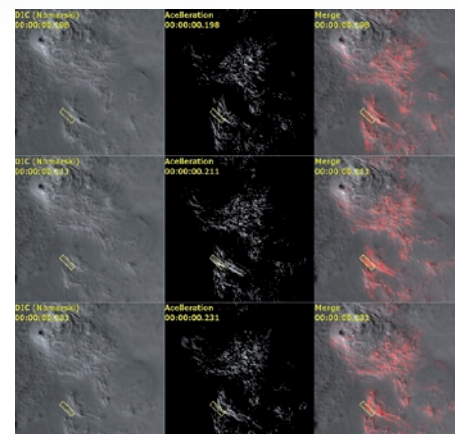


Fig. 1: The illustration shows frames taken at three times. Channel 1: DIC. Channel 2: time differential image. Channel 3: merged image. Channel 2 has been pseudo-colored-red. The yellow rectangle marks the region used for measurement.

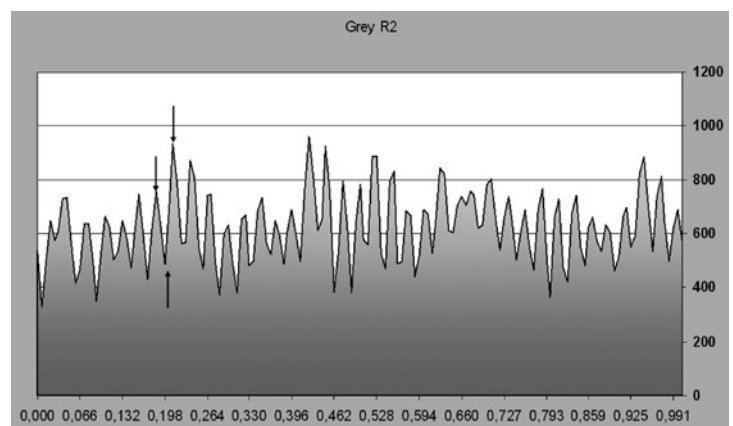


Fig. 2: Gray levels (Y axis) indicate the brightness fluctuations caused by the ciliary beat. The frequency shown is 39 beats per second. X scale in milliseconds. Arrows mark the representative images shown in Fig. 1.



while there are far more than 20 spectral versions of fluorescent proteins, some of which have outstanding quantum efficiency and stability. Smaller, synthetic fluorochrome molecules are also increasingly employed in live cells and tissues. This has equipped scientists with new tools for analyzing intracellular processes. To a growing extent, these tools are used to analyze processes of very high speeds.

### Fast in all Dimensions

#### The Time Dimension

Speed is of essence in several respects. First of all, the need for fast exposure times requires a fast camera that retains the advantages of digital camera technology over analog video technology to the greatest possible extent (e.g., better signal-to-noise ratio, higher sensitivity, greater dynamic range). An example of such a camera is the AxioCam HSm, whose 660x494-pixel sensor can be read out rapidly at 24.57 MHz. The camera features what is known as „streaming technology“, which permits simultaneous exposure and readout in fast time-lapse series of exposures. The AxioCam HS can thus accomplish frame rates from 60 fps (full frame) up to more than 200 fps (binning, ROI).

Figure 1 shows an application in which the beating frequency of the cilia of amphibian pulmonary epithelial cells (samples: Dr. Bob Hard, University of Buffalo) was examined with Nomarski differential interference contrast. The cilia of these cells beat about 30 times per second varying, depending on temperature. To obtain a sufficient number of frames for a more precise analysis, a binning factor of 2 for exposure and the camera limited to an area of 162 x 160 pixels. Under these conditions, and with an exposure time of 2 msec, 153 frames were recorded per second.

To permit quantitative analysis, second order differential images between consecutive time points were computed, which show only the acceleration component of the moving aspects in the image. This information is shown red in the

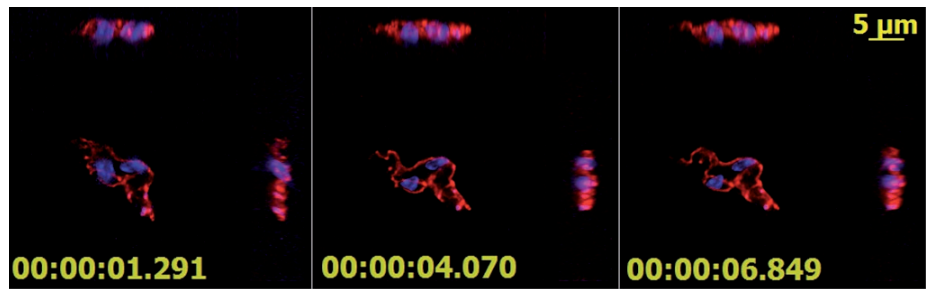


Fig. 3: Live trypanosomes, Z stack time series in two channels (Mitotracker Red, DAPI). Z stack deconvolved by constrained iterative deconvolution with spherical aberration correction. The frames represent three time points of time in a maximum projection in XY, YZ and XZ views.

merged image. It is easy now to measure the intensity, and to determine the beat frequency from it (Fig. 2).

#### The Wavelength Dimension

For fast exposures in several fluorescence channels, it is necessary to rapidly switch between the excitation wavelengths. The internal, motor-driven fluorescence filters are too slow for the purpose. Available for the new Cell Observer HS concept are two alternative xenon light sources, which allow switching between channels within a range of 1 to maximally 5 msec. Maximum Accordingly, the microscope is equipped with efficient multiple-bandpass beamsplitting and emission filters. Up to five channels can be configured for such an experiment.

#### The Z Stack Dimension

Given the continuing demand for higher spatial and temporal resolution, it has become necessary to record processes in three dimensions. This can best be accomplished by means of piezo-based focusing devices, which permit focusing with sufficient speed and precision. Two solutions are available for the Cell Observer HS: one is a stage with motorized X and Y axes and a plate that can be focused by means of piezo elements, and the other is a piezo unit that can move an objective along the Z axis.

In conjunction with fast excitation switching and suitable exposure times, these devices allow up to 30 Z planes to be recorded in two channels per second. In this way, fast processes can be captured reliably in three dimensions.

Figure 3 shows a live trypanosome during cell division recorded in two channels. These cells move at high speed by means of an undulating membrane. In the experiment shown, Z stack images were taken in 30 planes and in two channels (Mitotracker Red, DAPI).

#### Exact Timing

To avoid motion blur or light intensity fluctuations in fast multichannel image acquisition, the functions of the hard-

ware components involved need to be exactly timed. The use of trigger signals allows switching between the excitation wavelengths, Z positioning and timing of the exposures to be synchronized very precisely.

#### Processing

Z stack images acquired with an epifluorescence microscope frequently lack proper contrast, which is due to light originating from regions above and below the focal plane. By means of 3D deconvolution procedures, part of this out of focus light can be returned to its origin. For the Cell Observer HS, an additional iterative algorithm has been introduced, which permits the fast processing of Z stack time series. In addition to processing with a theoretically determined point spread function (PSF), now it is possible also to use measured PSFs.

#### The Aim: Acquisition of Measured Data from Images

As long as the acquisition of multidimensional images was dominated by technical problems, the aim primarily was to obtain "nice pictures" for presentation and publications. As these problems are getting solved, the interest increasingly centers on the acquisition of reproducible measurement results. For this purpose, the Cell Observer HS offers many capabilities, which can be combined into solutions matching the respective tasks. No matter whether these are simple interactive measurements (intensity vs. time) or more complex automatic multichannel measurements, or involve the capabilities of the new Colocalization module: quantitative data can be obtained from the images easily and reliably.

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