



Fig. 1: High Speed CCD Camera

# Digital High Speed Imaging for Microscopy

## Looking at Rapidly Moving Objects

In the past, the design of digital cameras for applications in light microscopy was driven by the demand for high resolution and sensitivity. As the demands for live cell imaging evolve we find that speed is also an increasingly important factor in microscopic observation. New high speed cameras combined with a suitable system environment cope with these demanding tasks and enable scientists to push the limits further.

### Video Camera Technology Is Obsolete

The recording of fast moving objects has been a remaining application of video camera technology in microscopy. Depending on the video standard used, it delivers 30 frames/s (NTSC), or 25 frames/s (PAL), however, the image quality achieved is far from sufficient. For example, video cameras clearly have problems in resolving weak differences in sample brightness and suffer from a low dynamic range. Furthermore, the interlaced readout method used by video cameras results in horizontal distortions when looking at fine, rapidly moving structures.

### Clear Benefits of Digital Cameras over Video Technology

- better contrast differentiation due to higher dynamic range combined with 12 bit digitisation
- higher sensitivity in low light situations due to low readout noise

- no need for frame grabber cards as standardised direct digital data transmission is used
- direct availability of movie sequences on the computer for further processing and export to presentation formats (AVI, MOV...)
- greater flexibility to adapt to different imaging situations
- on-chip pixel binning for further increase of sensitivity and speed
- sensor sub-region readout for reducing data volume and increase of speed
- adjustable exposure time allows flexibility of choice between speed and signal intensity
- trigger signals to synchronize acquisition to other components

### High Temporal Resolution Suited to Application Needs

Carl Zeiss MicroImaging expanded its range of digital AxioCam cameras with

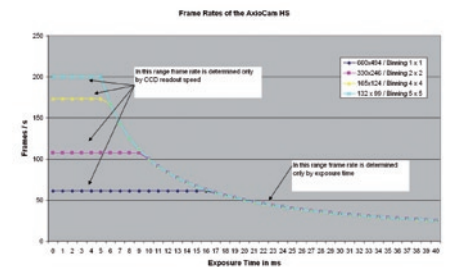


Fig. 2: Decoupling exposure and sensor readout by overlapping enables high frame rates, [www.zeiss.de/axiocam](http://www.zeiss.de/axiocam)

color and monochrome models specifically designed for high speed image acquisition. They offer all the advantages listed above and add further benefits.

The AxioCam HS captures image sequences at the same resolution as video technology but at more than twice the speed. This means, that moving objects can be recorded at 60 frames/s with a resolution of 660 x 494 pixels and by using binning and sensor sub-region readout, the frame rate can be increased to more than 300 frames/s.

The key feature is the use of a progressive scan VGA sized CCD-sensor which gives a very short readout time of 16ms thus enabling this high frame rate without interlace artefacts. For many applications in live cell imaging the use of lower resolution sensors is not a real drawback, as higher magnification lenses are generally used. For example we can

easily calculate that the useful resolution of a 63x NA 1.25 Oil lens requires an effective pixel count of only a 1/2 inch VGA CCD-sensor.

### AxioCam HS Embedded in an Optimized Fast System Environment

To support high speed acquisition with the AxioCam HS, an optimized module has been developed for Rel 4.5 of the AxioVision software package. It employs a system architecture which guarantees equidistant image time sampling points by streaming raw image data directly to the hard disk, without a bottle neck in the operating system. In addition, all image processing (compilation of the raw images into the ZVI image format, shading correction, high quality color interpolation, etc.) is decoupled from the acquisition process and performed after all time critical tasks have been completed. For optimum image quality, particularly in low light situations, no image compression is applied. The average data rate is approximately 28 Mbytes/s thus a recorded image sequence of 2.7 minutes fills a standard DVD-ROM.

Specific time lapse image editing and processing functions available in AxioVision Rel. 4.5 will allow part of a recorded sequence to be exported in common digital movie formats for direct import into PowerPoint presentations. In addition image analysis modules for time lapse processing, tracking and ratio imaging are available.

### High Speed in Multi-dimensional Cell Observation

Carl Zeiss MicroImaging offers with the "Cell Observer" system solutions for the automatic acquisition of multi-dimensional images. This concept has now been enhanced with fast components controlled by AxioVision software. High speed components such as wavelength switching light sources or Z-positioning piezo-devices, enable the capture of multi-dimensional image sequences at

high speed at the push of a single button.

The AxioCam HS is optimized for this environment by reducing the communication overhead as follows:

- Five exposure time registers can be preloaded with channel specific exposure times to minimize channel switch time
- Camera trigger input and output signals are used within this environment to synchronize the camera with the other components.

This means that up to 30 Z planes can be recorded in two fluorescence channels per second thus fast cellular processes can be captured reliably in three dimensions with minimized motion blur.

#### AxioCam HS Features:

- CCD basic resolution 660 (H) x 494 (V)
- Progressive sensor readout
- 1/2" CCD Sensor with 9.9 µm x 9.9 µm pixel size
- From 60 frames/s up to more than 200 frames/s
- Dynamic range typical SNR 65 dB = 1 : 1800
- Readout clock speed 24.57 MHz
- Digitization 12 bit AD converter
- Exposure time from 1 ms up to 60 s
- Overlapping exposure and readout modes
- FireWire Interface (IEEE 1394 a)
- Color and Monochrome version available

### Documentation of Protozoans in Termite Guts

The AxioCam HS was presented to the researchers at the "Marine Biological Laboratory" in Woods Hole, Massachusetts, USA. This group included Professor Jared R. Leadbetter from the California Institute of Technology. In his opinion, the AxioCam HS represents a considerable leap forward for research into living single-celled organisms, offering the user new standards of speed and image quality.

It was possible to demonstrate the AxioCam HS benefits directly during the observation of symbiotic single-celled organisms in the hindguts of termites.

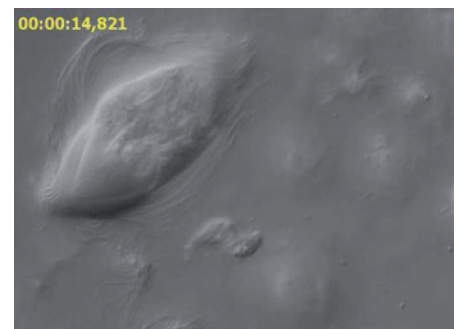
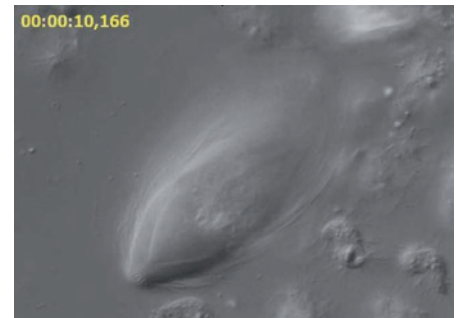
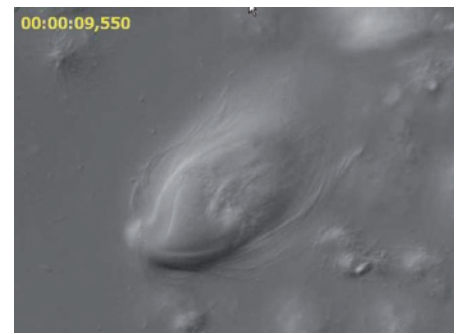


Fig. 3: Termite gut protozoan acquired with 40x/0.75 EC Plan-Neofluar with DIC using an Axio Imager and AxioCam HS, recorded at 55 frames/s, (showing different time points of the movie). Sample movies of termite guts protozoans: <http://www.zeiss.de/axiocam> → AxioCam HS → Gallery

Prof. Leadbetter was impressed. He said: „In DIC we always came up against the problem of hazy, blurred images when working with video cameras. The new AxioCam HS, on the other hand, impressed us by producing the best movies we have seen of these organisms.“ He adds: „It is really exciting to be able to observe these microbes in such a high temporal resolution and with such high dynamics. With this image quality it will be possible to make meaningful, quantitative measurements“.

#### Overview of possible application areas

| Subject Area           | Comments   |
|------------------------|--|
| Ecology                | Determination of water quality by motility analysis of protozoans.                                   |
| Protistology           | Determination and documentation of protozoans by analysing movement parameters.                      |
| Developmental Genetics | Observation of proteins coupled to fluorescent proteins in rapidly moving living organisms.          |
| Developmental Biology  | Ideally suited in combination with the new SteREO Lumar.V12.   |
| Physiology             | Intravital microscopy, capillary microscopy, blood flow analysis, cancer research                    |
| Inorganic Chemistry    | Formation and temperature behaviour of compounds (chemical structure and changes). Polymer research. |
| Reproduction Medicine  | Fertility analysis by observation and analysis of sperm motility.                                    |

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