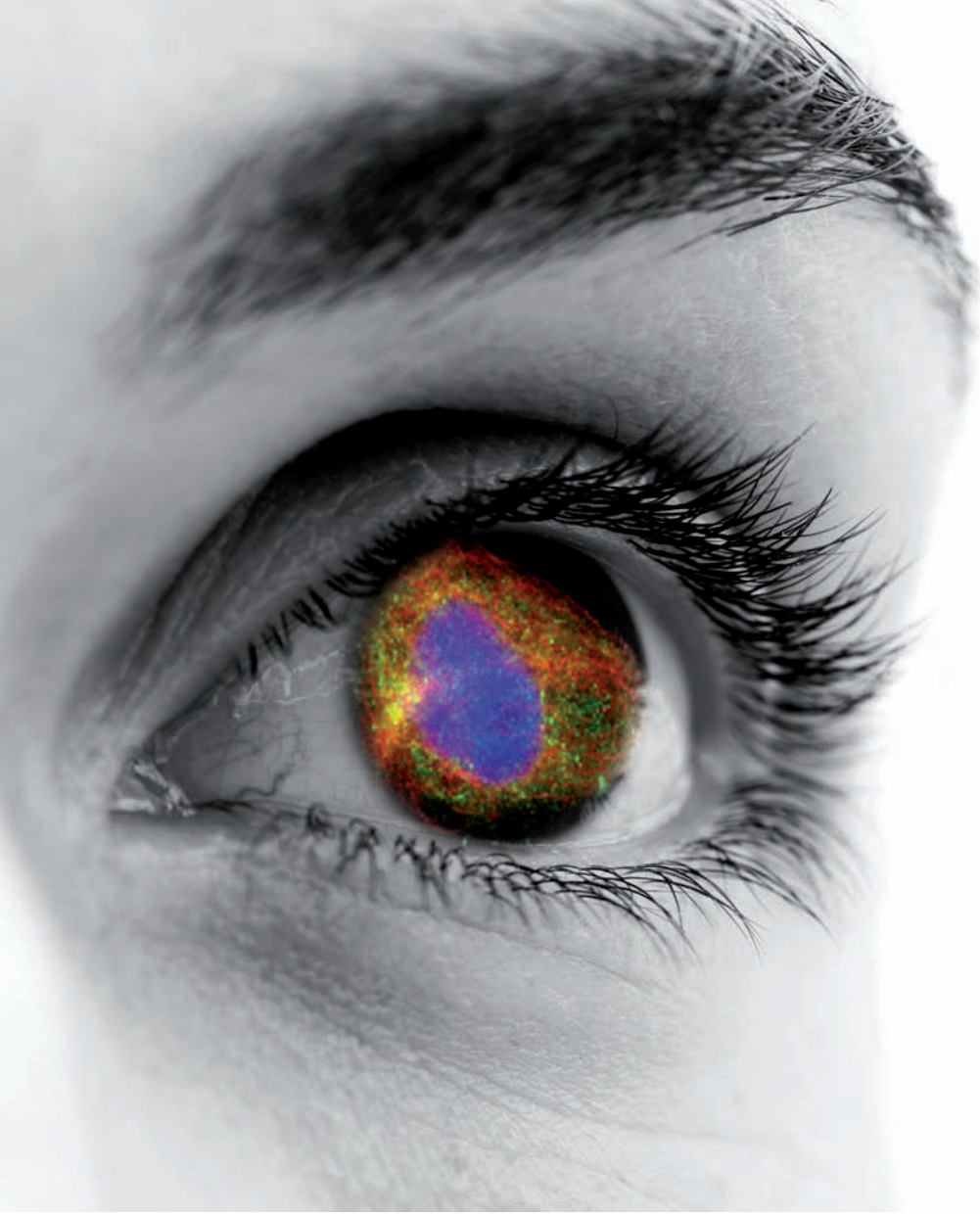


Leica DMI6000 B with Adaptive Focus Control

Explore Life in All Dimensions

Living up to Life

Leica
MICROSYSTEMS

Adaptive Focus Control

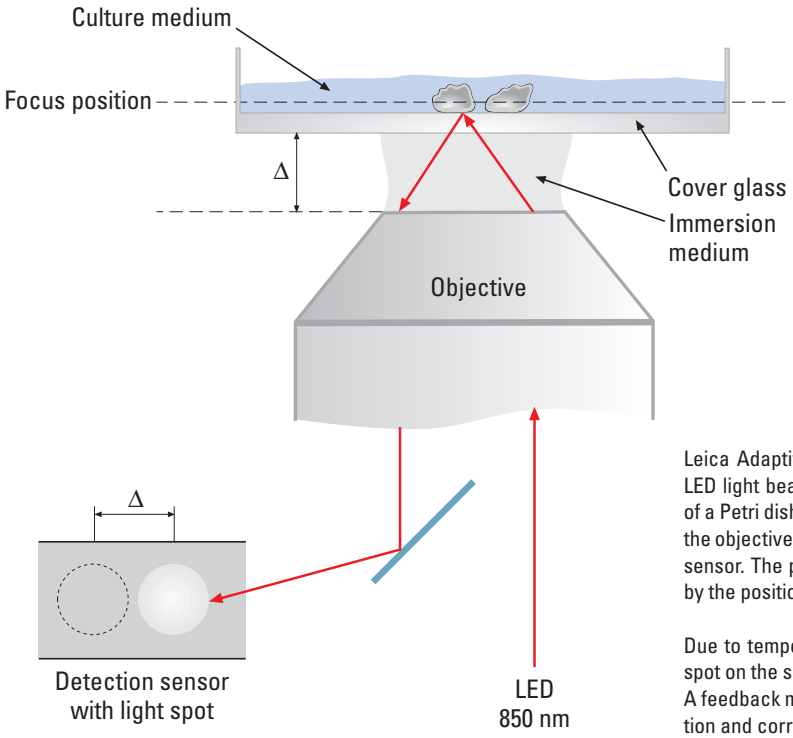
Imaging dynamic processes in living cells is challenging, even for the most precise and reliable microscopy systems. The goal is clear – to record, analyze, and publish changes observed over time, as accurately as possible, while keeping the specimen free of side effects arising from the observation itself.

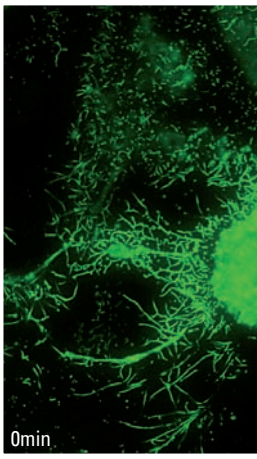
Improving the best:

The Leica DMI6000 B inverted microscope is the core component of Leica Microsystems' widefield and confocal systems for live cell imaging, offering unsurpassed stability inherent to its design. It is capable of effortlessly maintaining the focus under live cell imaging conditions over extended time periods. However, even the most robust microscope is susceptible to sudden temperature fluctuations, for instance if a climate chamber needs to be opened to add a solution to the sample during the course of an experiment. This can result in unsharp images in the time series, or even losing the sample out of focus completely.

Explore Life

Leica Microsystems has developed the DMI6000 B with Adaptive Focus Control (AFC) for researchers who demand consistent multi-dimensional imaging without loss of focus. Available for both widefield and confocal applications, the AFC dynamically regulates the focus position, whenever or wherever the experiment requires it. Tested and approved in collaboration with scientific partners, Leica's Adaptive Focus Control ensures that the specimen remains in focus throughout the experiment.



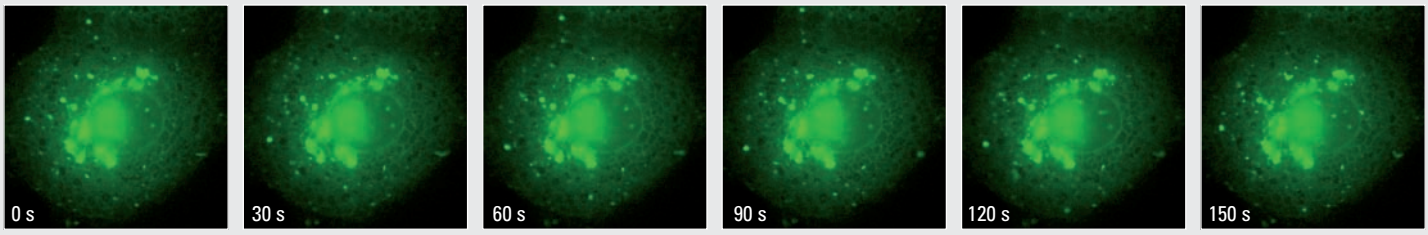


Leica Adaptive Focus Control is based on the reflection of a LED light beam at an appropriate surface such as the bottom of a Petri dish or multi well plate. The light is projected through the objective to the surface and reflected onto a light-sensitive sensor. The position of the light spot on the sensor is defined by the position of the sample in relation to the objective.

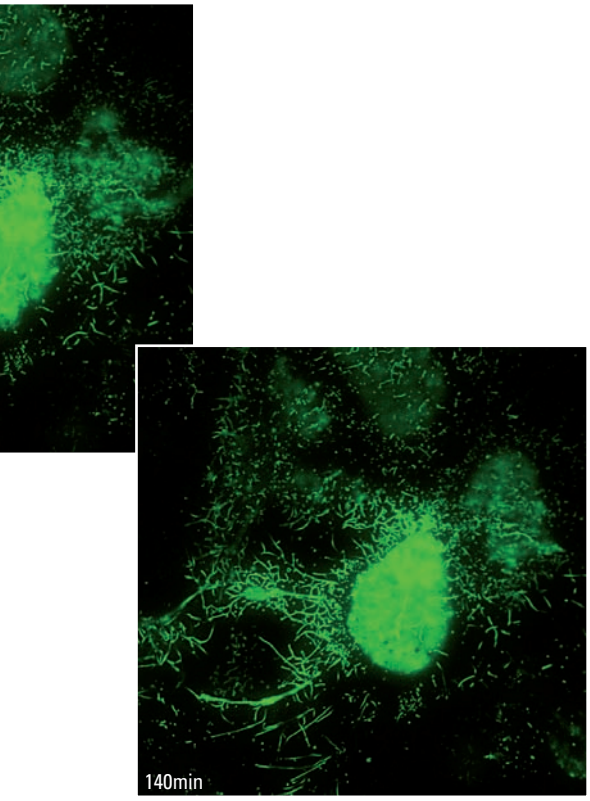
Due to temperature drift, this distance changes and the light spot on the sensor is shifted in relation to the original position. A feedback mechanism records this shift in the light spot position and corrects the z-position accordingly.

in All Dimensions

AFC switched on



COS cells were transfected with a Golgi specific GFP variant. The microscope system was placed in a climate chamber at 37°C.
Shortly after starting the acquisition, 1 ml of an ice-cold salt solution (PBS) was added (60 s). Throughout the entire acquisition sequence, the sample remains in the desired focal plane. Courtesy of Prof. Dr. Ralf Jacob and Dr. Alexandra Elli, Institute of Cytobiology and Cytopathology, University of Marburg, Germany.



TIRF imaging with simultaneous AFC
MDCK cells expressing GFP-p75
Courtesy of Prof. Dr. Ralf Jacob and Dr. Alexandra Elli, Institute of Cytobiology and Cytopathology, University of Marburg, Germany.

When experience counts

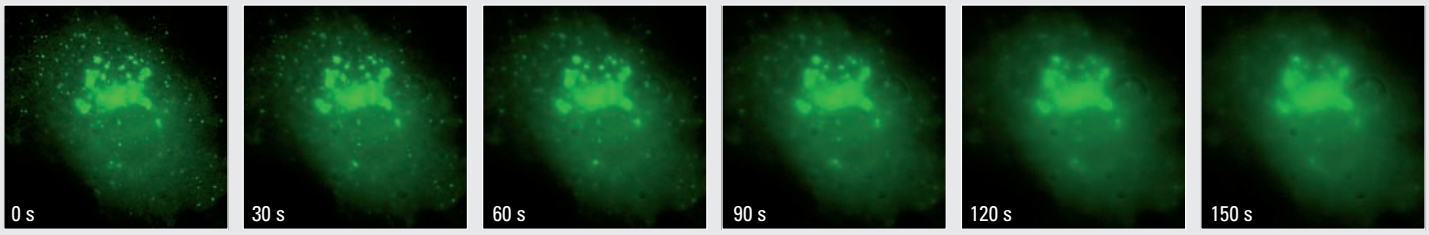
Leica Microsystems' experts designed the DMI series with great emphasis on reliability. Now, with the addition of the AFC, the microscope becomes the ultimate tool for live cell applications that demand fast reactions to changing conditions. Based on pioneering technology, the AFC principle involves the reflection of a light beam at a suitable surface to keep the distance between the objective and specimen constant. The process is established, robust, and remarkably fast.

Smoother integration

To be truly effective for a wide range of experiments, Adaptive Focus Control is integrated into Leica Microsystems' intuitive software workflow. For full flexibility, AFC can also be operated in stand-alone mode without PC connection using the microscope's function keys. Remote control is possible via the Leica SmartMove or Leica STP6000 control panel. No separate control devices are needed. The underlying technique works for all selected contrast methods and does not require taking additional images of the specimen. This ensures that cells remain viable for longer, and deliver reliable results over long periods of time.

The Intelligent Automation of the Leica DMI6000 B offers ultimate ease of use. A single push of the 'hold focus' button is all it takes – the system works automatically in the background, optimizing the results for each objective. The system focuses on the specimen, while you focus on getting results.

AFC switched off



COS cells were transfected with a Golgi specific GFP variant. The microscope system was placed in a climate chamber at 37°C.
Shortly after starting the acquisition, 1 ml of an ice-cold salt solution (PBS) was added. Focus drift can be clearly observed when the cold solution is added (60 s).
Courtesy of Prof. Dr. Ralf Jacob and Dr. Alexandra Elli, Institute of Cytobiology and Cytopathology, University of Marburg, Germany.

The need for speed

When moving from position to position within a specimen, small differences in focus are often observed as you look down the eyepieces. The impressive speed of the AFC is clearly demonstrated here, as it automatically corrects for these small focus differences in real time. This is of great benefit when imaging multi well plates.

Naturally, AFC can be used for fast process monitoring. Imagine you want to monitor vesicle movement in 3D over time. The challenge here is to keep the cells in focus while acquiring high-speed z-stacks. This is achieved with the Leica Super Z Galvo stage. You set up the time lapse experiment in combination with z-stacks, defining when and where AFC will be activated. The experiment benefits from faster, reliable results – no over-sampling, less stress to the specimen due to less light exposure, and smaller data sets.

Get to grips with multidimensional space

Multidimensional experiments are the backbone of live cell imaging – the ability to visualize multiple locations in 3D over time is of paramount importance.

Focusing on change

Cells are dynamic structures – they change shape constantly and even round up before entering mitosis. What is of interest and sharply in focus right now may be at a completely different z position a couple of hours later. Here you need the flexibility to dynamically adapt to changing cell positions or morphology. The solution is simple: the combined action of AFC with the Leica digital autofocus for extra focusing versatility. The possibility to define exactly when and at which positions this focusing combination is activated ensures every event in the time lapse is captured.

Leica DMI6000 B with Adaptive Focus Control – your reliable system solution for live cell imaging. Time saving and improving results.

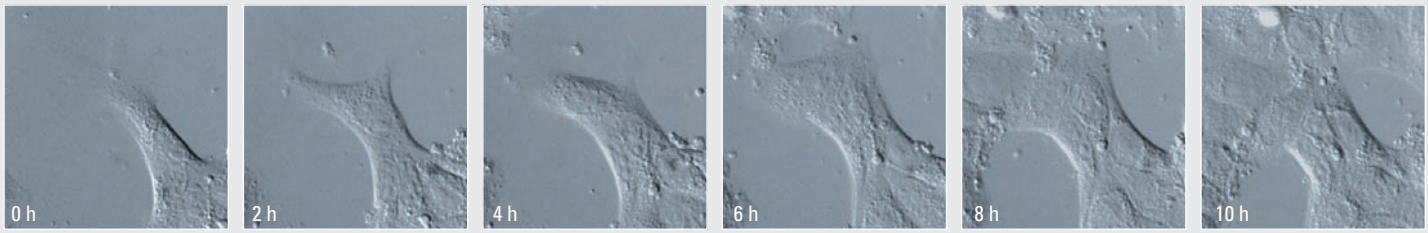
"AFC is such a powerful tool for time lapse experiments. I am impressed how fast and reliable it is."

Prof. Dr. R. Jacob
University Marburg,
Department of Clinical Cytobiology and Cytopathology, Marburg, Germany

For best publishable results:
Building on the proven stability of the Leica DMI6000 B, Adaptive Focus Control keeps the specimen actively in focus – whenever and wherever the experiment requires it.

- **Uncompromising speed**
- **Utmost compatibility**
- **Unrivaled ease of use**

in All Dimensions



Time series with DIC contrast method
MDCK cells (Madin-Darby Canin Kidney) were imaged over a time period of 10 hours. AFC was switched on.
Courtesy of Prof. Dr. Ralf Jacob and Dr. Alexandra Elli, Institute of Cytobiology and Cytopathology, University of Marburg, Germany.

Technical Specifications

Systems with Adaptive Focus Control	Leica DMI6000 B with AFC, AM TIRF MC, TCS SP5 II with AFC, TCS SP5 II DS with AFC; TCS SP5 MP with AFC
Suitable specimens	Living cells in cell culture (embedded specimen only with dry objectives)
Suitable dishes, coverslips	Glass bottom dishes and multi well plates, N 1.5 (0.15- 0.18 mm thick), specified plastic dishes*
Detection light	850 nm LED
Sensor	CMOS sensor
Control via	Function keys on the microscope; SmartMove, STP6000 and application software
Operation modes	Stand alone (without software) and integrated in software workflows
Supported software	Leica LAS AF as well as 3rd party software*
Supported objectives	Please contact your local sales representative for the updated objective list
Memory	Via software: unlimited AFC hold positions (in combination with LAS AF Mark & Find)
Focusing	Via motorized z drive with travel range of 8 mm (2 mm below, 6 mm above the stage) smallest increment: 15 nm/step; with parfocality manager,
Operating environment	15 – 42 °C Relative humidity: 60%
Operating voltage	90 – 250 V, frequency 50/60 Hz

*Please contact your local sales representative for detailed information

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