From Eye to Insight

Leica
MICROSYSTEMS

NEUROSCIENCE SOLUTIONS

by Leica Microsystems

WORKING WITH ACUTE SLICE PREPARATIONS

The nervous system works as a tightly regulated, highly complex cellular network. Besides gaining knowledge of the single unit of the network, the neuronal cell, the understanding of the network as a whole is of major importance for completing the picture of neuronal communication. One approach for investigating neuronal network activity is to use thick slice preparations (around 300 µm) of neuronal tissue for research, as most of the axonal connections between the cells within the slice remain conserved in such preparations.

CONVENIENT SLICE PREPARATION WITH LEICA STEREOMICROSCOPES

The preparation of acute slices for performing live-cell imaging and/or patch-clamping is a very time-consuming process. Working with the delicate tissue slices demands much patience, experience and gentle handling of the sample to avoid disruption of the neuronal network by vibration or other physical stress. During the preparation process a reliable, precise and convenient-to-use instrument with outstanding optics like a Leica stereomicroscop helps to minimize stress for both, the specimen and the experimenter.

AVOID VIBRATIONS DURING THE EXPERIMENT

Avoiding physical stress for the specimen continues once the slice has been transferred to the actual patch-clamp setup. Here, any manipulation of the microscope (e.g. changing the objective, using sliders, focusing etc.) can disrupt the axonal connections within the slice or the acquired patch-clamp configuration. The Leica DM6 FS fixed stage microscope is especially designed for working with slice preparations. Due to its intelligent motorization it allows all microscope functions, from switching between contrast methods to changing magnification, to be operated without touching the microscope, resulting in vibration-free experiment conduction.

INTELLIGENT AUTOMATION SAVES TIME AND RESOURCES

The optimized automation concept therefore saves the sample from accidental disruption, which in turn saves time and resources. As all current-carrying elements are switched off if not in use, the Leica DM6 FS provides ultra-high electronic stability in concert with superior optics and mechanical stability. Combined with the Leica IRAPO HCX 25x 0.95 NA water immersion objective, which allows a magnification changer to be used instead of changing the objective, the Leica DM6 FS fixed stage microscope is the best choice for patch-clamping and live-cell imaging in slice preparations.

WORKING WITH ADHERENT CULTURED OR DISSOCIATED CELLS

There are two main reasons for patch-clamping or live-cell imaging of adherent cells – one is to gain information about the cellular physiology of a specific cultured or freshly dissociated cell type, the other is to gain information about the behavior of specific molecules within a physiological process e.g. an ion channel in a signaling cascade. For the first, the body of interest is the cultured or dissociated cell itself. For the latter, the cell type plays a minor role as the focus is on specific physiological processes that might occur in a variety of cells or might be mimicked in an easy-to-culture commercial cell line by transfection of a gene of interest.

CULTURED OR DISSOCIATED CELLS ARE THE PERFECT SAMPLE FOR LIVE-CELL IMAGING

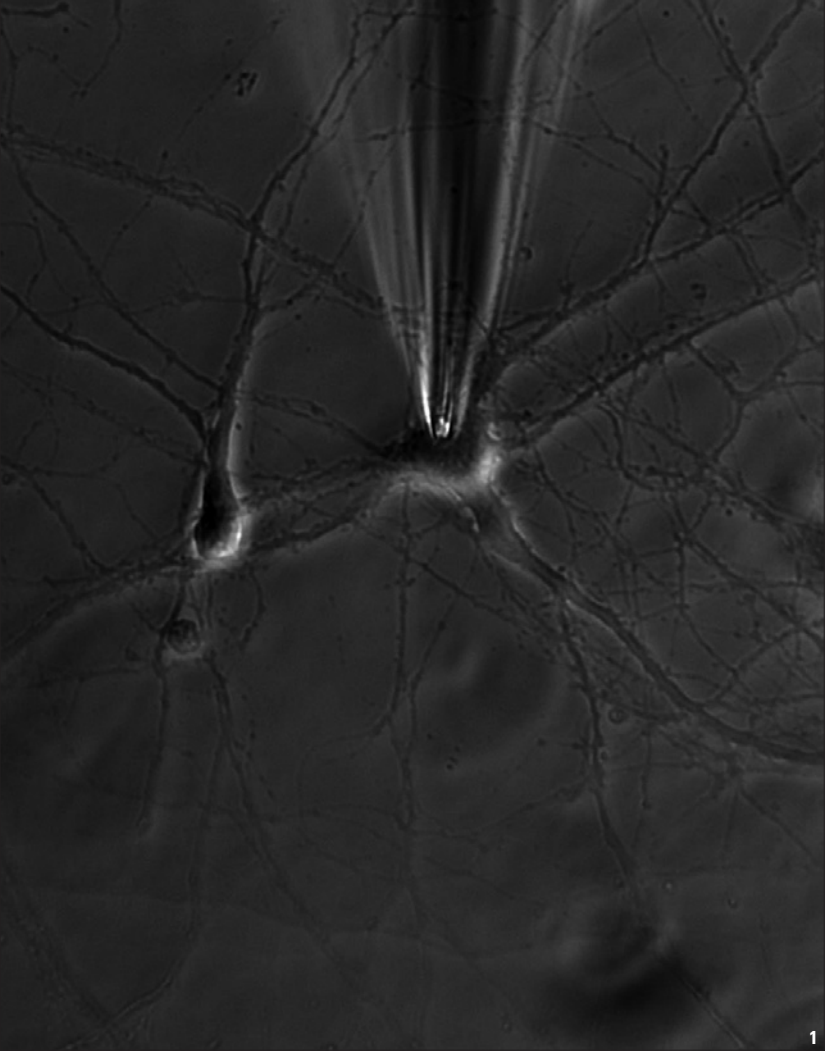
An advantage of using cultured (or dissociated) adherent cells for physiological investigations is that these cells usually exist in single cell layers and are easily accessible for perfused substances (e.g. inhibitors or activators of ion channels). Additionally, such specimens produce less stray light, which shortens the exposure time with fluorescence excitation light. This saves the sample from photobleaching and harmful irradiation, which is advantageous for both high-speed imaging and long term live-cell imaging experiments.

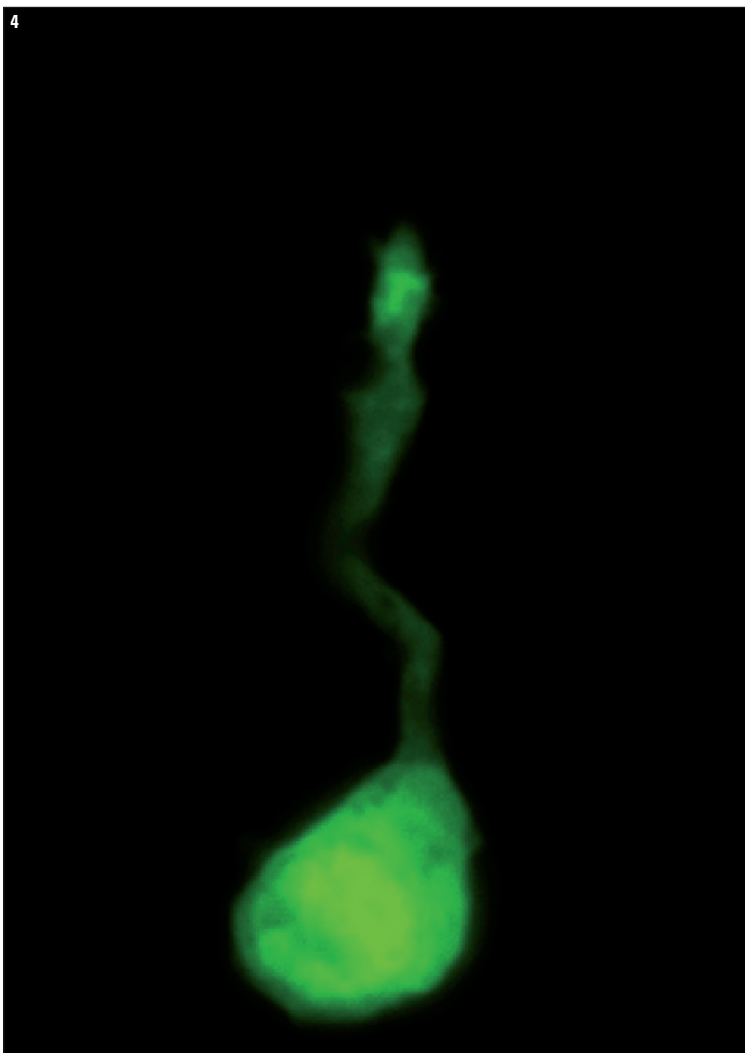
INVERTED MICROSCOPES ARE BEST SUITED FOR WORKING WITH ADHERENT CELLS

Cultured or dissociated cells are mostly kept in culture dishes. This means that inverted microscopes can be used to perform live-cell imaging or patch-clamp experiments. Inverted microscopes are convenient for working with culture dishes and offer a lot of space around the sample, enough for the placement of multiple micropipettes, suctions, perfusion needles and other equipment. Additionally, the cells are easily accessible for patch-clamp pipettes to perform electrophysiological experiments.

DESIGN YOUR MICROSCOPE SETUP

Leica Microsystems offers a range of inverted microscopes from non-motorized versions for routine applications through fully coded and partially motorized microscopes for advanced applications to fully automated versions for high-end applications like combined patch clamping and high-speed multi-channel live-cell imaging. Peripheral equipment such as superfast external filter wheels (wavelength switching in less than 24 ms!) and tailor-made climate chambers make your live-cell imaging microscope a high-end setup for top-notch research.



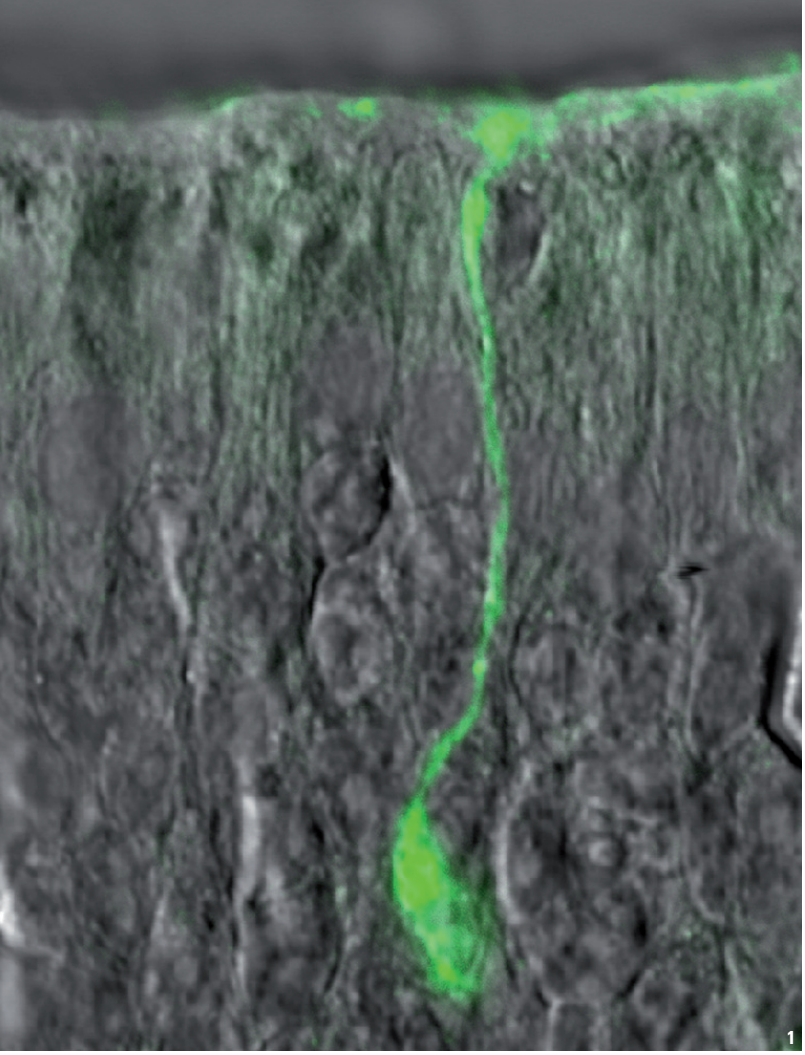


1 Phase contrast image of a hippocampal neuron in a co-culture with astrocytes. The patch-pipette is attached to the cell ready for recording. Image courtesy of Dr. Ainhara Aguado, Ruhr-University Bochum, Germany.

2 The Leica DMi8 series of inverted microscopes are the perfect tools for performing electrophysiology and live-cell imaging with cultured or other adherent cells. With models ranging from non-motorized to fully-motorized versions, all demands are covered. Combined with equipment like Leica fast filter wheels, tailor-made incubation chambers, motorized stages and the user-friendly and powerful LAS X software, you can build up the patch-clamping and/or imaging setup that perfectly suits your requirements.

3 Leica Fast External Filter Wheels allow rapid vibration-free changing of the wavelength within 24 ms (adjacent positions), making them the fastest filter wheels available on the market. Four filter wheels with five freely definable positions can be employed simultaneously on both the excitation and the emission side for maximum flexibility and a great number of different applications. As all of our imaging components (cameras, filter wheels, shutters) are synchronically operated by special sequencer boards via the LAS X software, we offer ultra-high imaging speed with just a few mouse clicks.

4 GFP-labeled acutely dissociated olfactory sensory neuron. Image courtesy of Dr. Jennifer Spehr, RWTH Aachen University, Germany.



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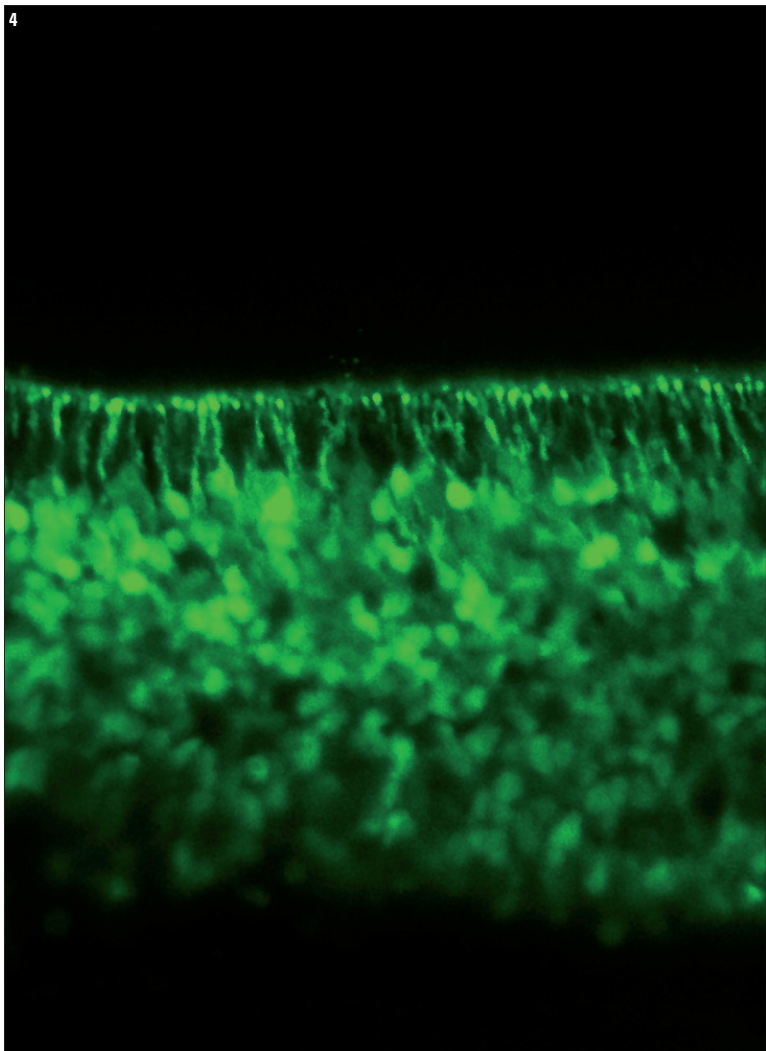

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1 GFP-marked olfactory sensory neuron in an acute slice of mouse main olfactory epithelium (in differential interference contrast). Image courtesy of Dr. Daniela Flügge, RWTH Aachen University, Germany.

2 The Leica DM6 FS fixed stage microscope is a fully automated high-end research instrument suitable for sophisticated imaging experiments besides electrophysiological work. With its intelligent automation concept all operations of the microscope (e.g. ob-jective/magnification changing, focusing, switching between contrast methods) can be performed during an ongoing experiment without causing any vibrations. As all current-carrying elements and motors are turned off if not in use, the Leica DM6 FS fixed stage microscope helps to eliminate electrical noise in your patch-clamp setup.

3 The Leica HCX IRAPO L 25x/0.95 W high-end water immersion objective is especially designed for electrophysiological applications and near-infrared DIC. It combines a relatively low magnification (25x) with a high numerical aperture (0.95), which allows the use of a magnification changer without getting empty magnification. Hence, objective changing is virtually obsolete. Additionally, the objective has a very steep access angle (41°) and a very long free working distance (2.5 mm) which ensures convenient access of the pipette to the sample.

4 Confocal image of calcium dye Fluo-4 loaded cells in an acute slice of mouse main olfactory epithelium for live-cell imaging acquired with a Leica DM6000 CFS fixed stage microscope. Image courtesy of Dr. Daniela Flügge, RWTH Aachen University, Germany.

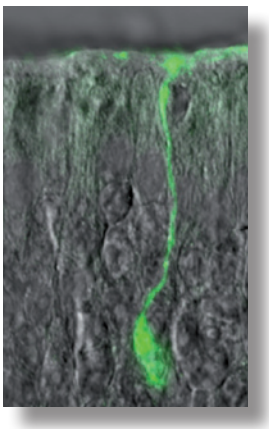
www.leica-microsystems.com

Leica MICROSYSTEMS

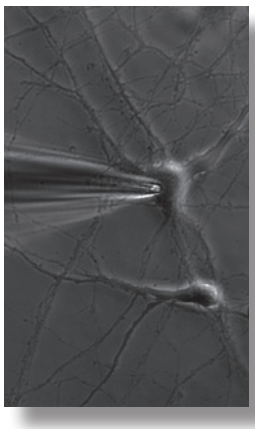
Fixed stage microscope

Stereo-microscope

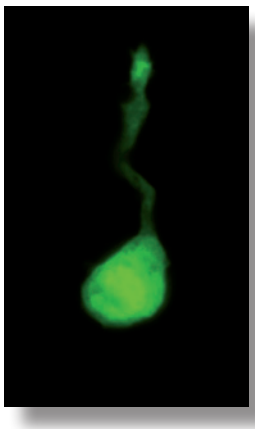
Inverted microscope



Acute neuronal tissue slice



Neuronal (co-) culture

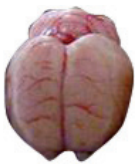


Acutely dissociated neurons

Transfection into a cell line

Tissue slicing/chopping

Vibrating microtome

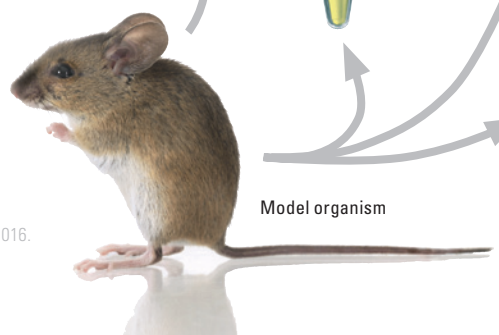


Tissue preparation

Cell preparation

Neuronal cell line

Extraction of gene of interest



Model organism