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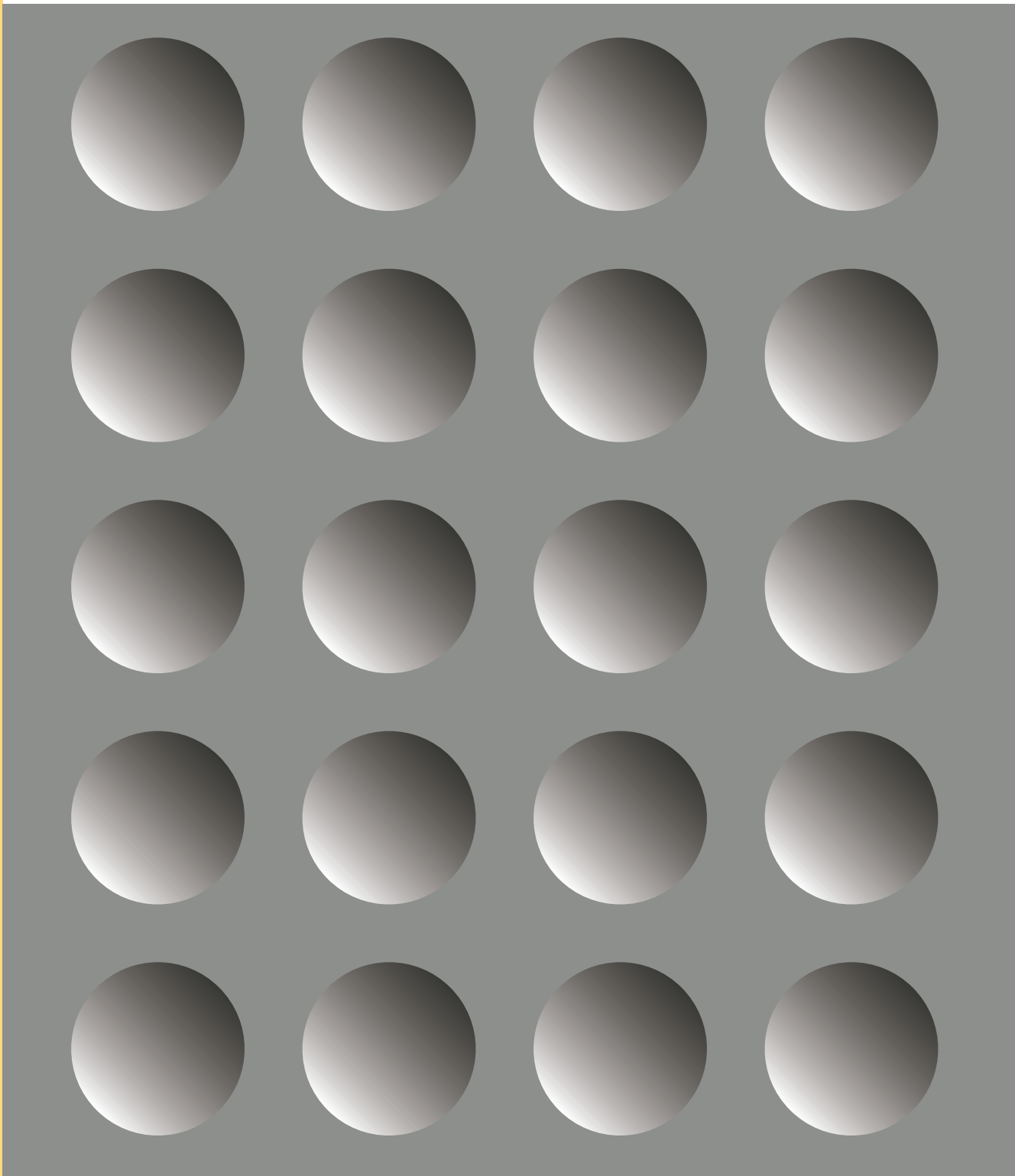
Innovations in Electrophysiology

robocyte2

Automated Voltage-Clamp Screening for *Xenopus* Oocytes



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Introduction

Oocyte Screening Goes Automatic

Oocytes of the toad *Xenopus laevis* are widely used as an expression system for ion channels, transporters, and receptors in drug development. *Xenopus* oocytes are big, robust cells (about 1 – 1.2 mm in diameter), can be obtained in large numbers, and are easy to handle.

Nevertheless, the low throughput of manually performed electrophysiology prevents its use for secondary functional screening of drug targets.

Ten years ago, Multi Channel Systems (MCS) offered the first commercially available fully-automated system for *Xenopus* oocyte screening, the Roboocyte. Now, it is time for the Roboocyte2, the new version of the Roboocyte:

The Roboocyte2 is a fully-automated all-in-one solution for high-throughput screenings of ligand-gated and voltage-gated ion channels, as well as electrogenic transporters based on the standard *Xenopus* oocytes expression system. All necessary tasks are accomplished by one single robot.

The automation of TEVC recording revolutionizes pharmaceutical drug discovery:

A much higher throughput can be achieved at greatly reduced costs.

- The general conditions of an experiment can be standardized, enhancing reproducibility.
- It allows your highly qualified personnel to do away with routine work and to focus on the experimental design and the analysis of collected data.
- The Roboocyte2 can be operated overnight without supervision. You can start an additional experiment at the end of a working day and analyze the results the next morning.

The Roboocyte2 was designed not only for those familiar with the *Xenopus* oocyte expression system, but to also encourage others to step into an exciting and new technology in drug discovery.

Features

- TEVC recording of voltage-gated and ligand-gated ion channels and electrogenic transporters
- Flexible design of automated recording sequences
- Automated cell wash
- Automated compound application

Advantages

- 24 h operation
- Plug and play
- Easy to use
- Cost effective
- Time saving
- High throughput


Welcome to

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Hardware

Smart, Compact, and Easy-to-use System

The Roboocyte2's compact and functional design saves space on your work bench. It is compatible with standard lab equipment and can be easily integrated in your working environment. Software controls for setting the pressure or adjusting the amplifier replace any knobs on the device. The Roboocyte2 is straight forward and easy to operate; handling does not require special skills or special equipment.

The recording is performed using disposable standard 96-well plates, which are commercially available from several providers. The oocytes are plated into the wells in a couple of minutes. They quickly settle within the cone-shaped wells and adhere to the well bottom after a few hours. The oocytes do not have to leave the plate anymore; you can easily transfer the oocytes from the incubator to the Roboocyte2 device and back again.

The well plate carrier, powered by linear motors, hovers smoothly and noise-free on a cushion of pressurized air above a magnetic steel plate. It operates at 20 µm resolution. The complete system does not require maintenance other than occasional cleaning of the steel plate.

The vertically moving z-arm, holding the TEVC probe, is designed for the high demand of speed and precision. The z-axis moves at a resolution of 20 µm; position and speed are computer-controlled. A quick adjustment process guarantees the precise impalement of the oocytes. We recommend using the ready-made measuring heads provided by Multi Channel Systems. If you prefer to produce your own, it is strongly recommended to use blank measuring heads from MCS. You may then fix your own capillaries, perfusion tubing, and silver wire to the mounting support.

The "ClampAmpC" high performance amplifier is specifically designed and manufactured for the two-electrode voltage clamp method by MCS hardware specialists. Ready to use TEVC probes make the handling quick and easy. The Roboocyte2 package includes everything you need to start right away, including a high performance computer with monitor, TEVC probes, tubing, the Roboflow liquid handler, and accessories.

Features

- Sequential recording of 96 oocytes without user intervention
- Neither special skills nor special equipment required
- Easy oocyte handling in disposable standard well plates
- Maintenance-free system
- High performance TEVC amplifier
- Support of the Roboflow liquid handler or a standard Gilson liquid handler

Hardware Details

Flexible and Automated TEVC Recording

Once the recording run has started, it proceeds automatically for all 96 oocytes, or for the selected oocytes without supervision. The recording sequence of each single oocyte can be flexibly designed exactly to your requirements. You can include reference values to trigger recording sequences, depending on the response of the oocyte. Automated controls identify unhealthy oocytes to eliminate unnecessary recordings. This means throughput is maximized while compound usage is minimized. Results obtained with the Roboocyte in the laboratories of Bayer AG show that an amount of up to 60 compounds, each paired with one positive control, can be applied to a single good oocyte. In theory, you can test several hundred compounds on a single 96-well plate before user intervention. The amount of compounds tested is in no way limited by the Roboocyte2 – it depends only on the properties of the compounds and on the viability of the cells. This increases the screening throughput in a revolutionary way.

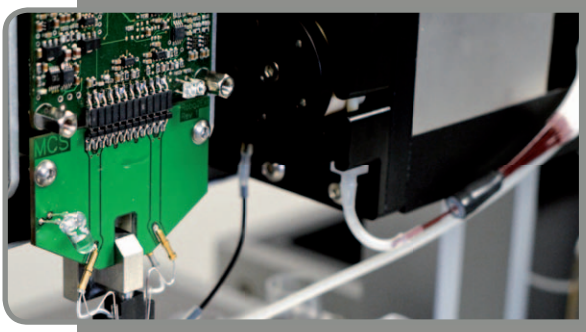
Ready to Use TEVC Probes

The impalement of the oocyte for recording is fast, precise, and gentle, thereby minimizing cell damage. Repeated impalements of a single oocyte are possible. After filling the glass electrodes with KCl solution, the supplied TEVC probes are ready to use. Simply connect the probe to the z-arm and the perfusion manifold and you are ready to go. The probe can be stored overnight and reused for several days.

The perfusion inlet leads to either a manifold that is connected to the Roboflow pinch valve system or to the external liquid handler Gilson GX-271. A peristaltic pump aspirates the fluid via the outlet and leads it to the waste receptacle. The probe design ensures a steady and pulse-free flow.

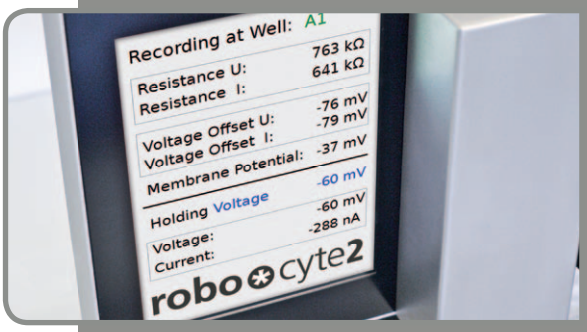
ClampAmpC

The small-sized high performance digital amplifier is integrated within the Roboocyte2 robot. It operates either in current clamp or voltage clamp mode and uses a PI based technology. The ClampAmpC records up to ±107 µA at a resolution of 1 nA. It is fully computer controlled. Features such as automated check of electrode resistance and automated clamp optimization are part of the Roboocyte2 software.



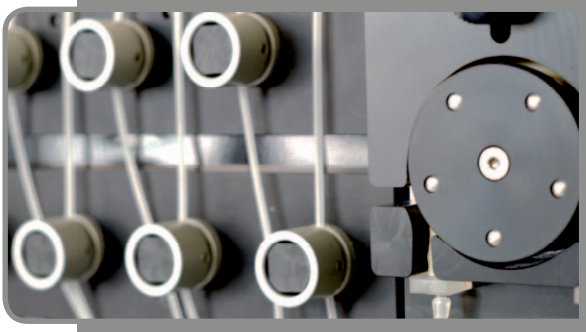
Status Display

The integrated LCD display shows all relevant parameters such as electrode voltage offsets, electrode resistances or the membrane potential of the cell after impalement.



Roboflow Perfusion System

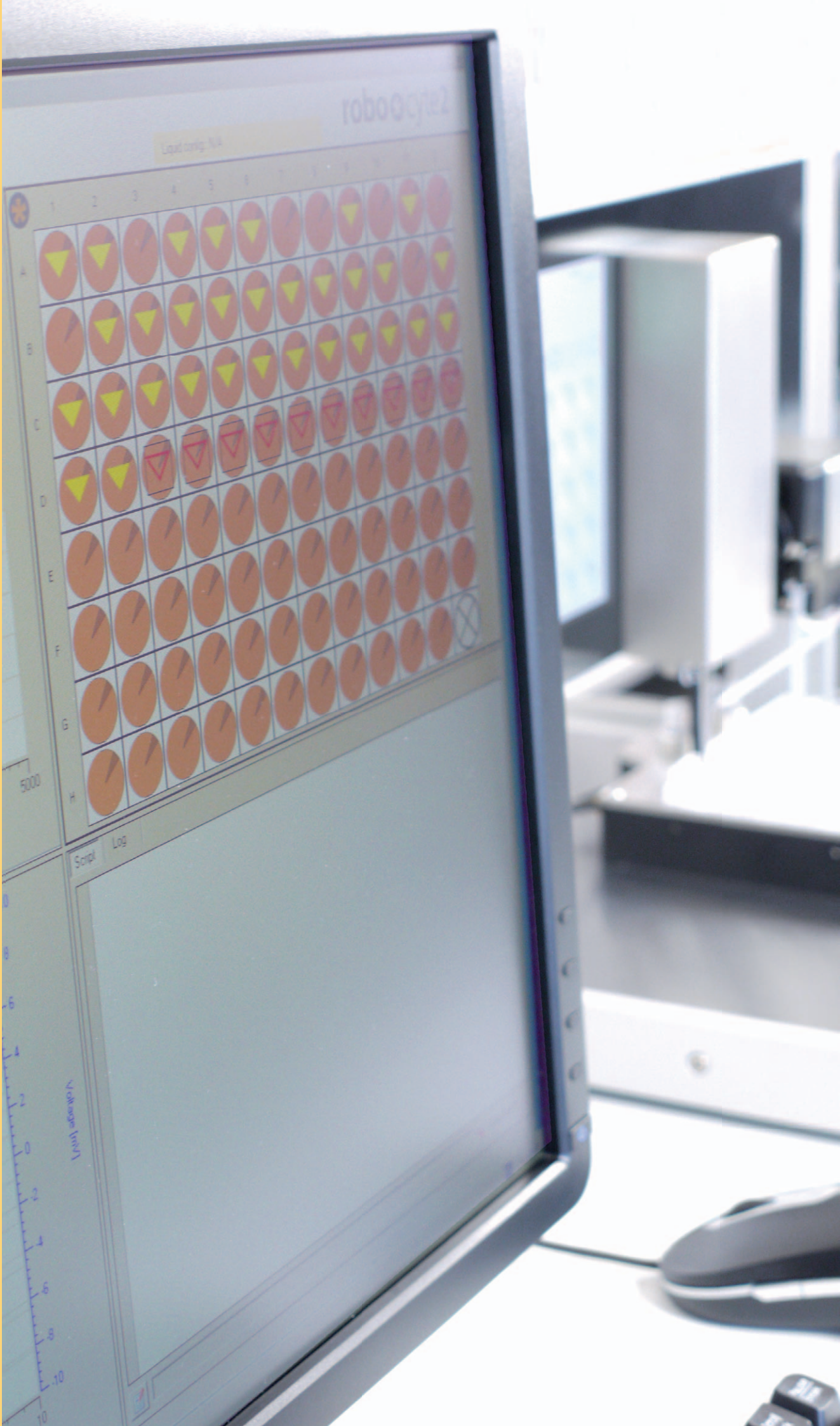
The perfusion with the Roboflow-System is simple and easy to use and is ideally suited for most rapid standard tests, dose response analyses, and small screens. A system with twelve valves and two peristaltic pumps is available. For more demanding tasks, such as a high-throughput compound screening, the Roboocyte2 easily interfaces with widely used standard liquid handler Gilson GX-271.



Automated Compound Application

The perfusion is continuous, but can also be paused during the recording process. The small volume of a well ensures that a flow rate of about 3 - 4 ml/min achieves a rapid and efficient fluid exchange with minimal compound usage. You can easily implement your own automated drug-saving strategies into your experimental setup, for example, testing the viability of an oocyte automatically each time before delivering a compound.

If you use either the integrated twelve pinch valve Roboflow-System or the external liquid handler Gilson GX-271, all perfusion protocols are automated and controlled by the Roboocyte2 software.


Script
Log

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Software

Flexible and Intelligent Solution

Oocyte impalement, compound application, TEVC recording, and online analysis are all performed automatically under computer control. The main characteristic and the main advantage of the Roboocyte2 is its full automation. You design the experiment and define all parameters in advance. At run time, you start the session with a single mouse click. The Roboocyte2 controls the recording for all 96 oocytes in a well plate automatically, even including a wash cycle. Thus the recording can go on 24 hours a day, unsupervised.

The easy-to-use graphical user interface of the Roboocyte2 software makes daily work with the Roboocyte2 quick and easy. Automated software controls replace any knobs on the robot. Customizable response-dependent recording sequences and control routines replace personal supervision.

How is this high degree of automation for such a demanding task achieved?

This is realized by java scripts, small text files containing commands. Test scripts and scripts for standard experiments are provided by Multi Channel Systems. Each user can write own scripts with the included editor. All experimental settings are defined within the script. You can write a script for any kind of experimental setup. Once the appropriate script is loaded into the Roboocyte2 software, simply click the "Start" button to start the robot. The script is then performed without the need for further customization and supervision.

The Roboocyte2 Java Scripting Language includes all important commands and functions in a nutshell. This concept makes the Java Scripting Language sophisticated and powerful, but not redundant or unnecessarily complex. The structure and command names are designed to be very intuitive and user friendly. You do not need any programming skills; a very basic understanding of the general concept is sufficient to experience the full power of scripting.

For quick tests, a convenient manual mode is provided as well. You can manually control all Roboocyte2 actions and parameters that are otherwise automated.

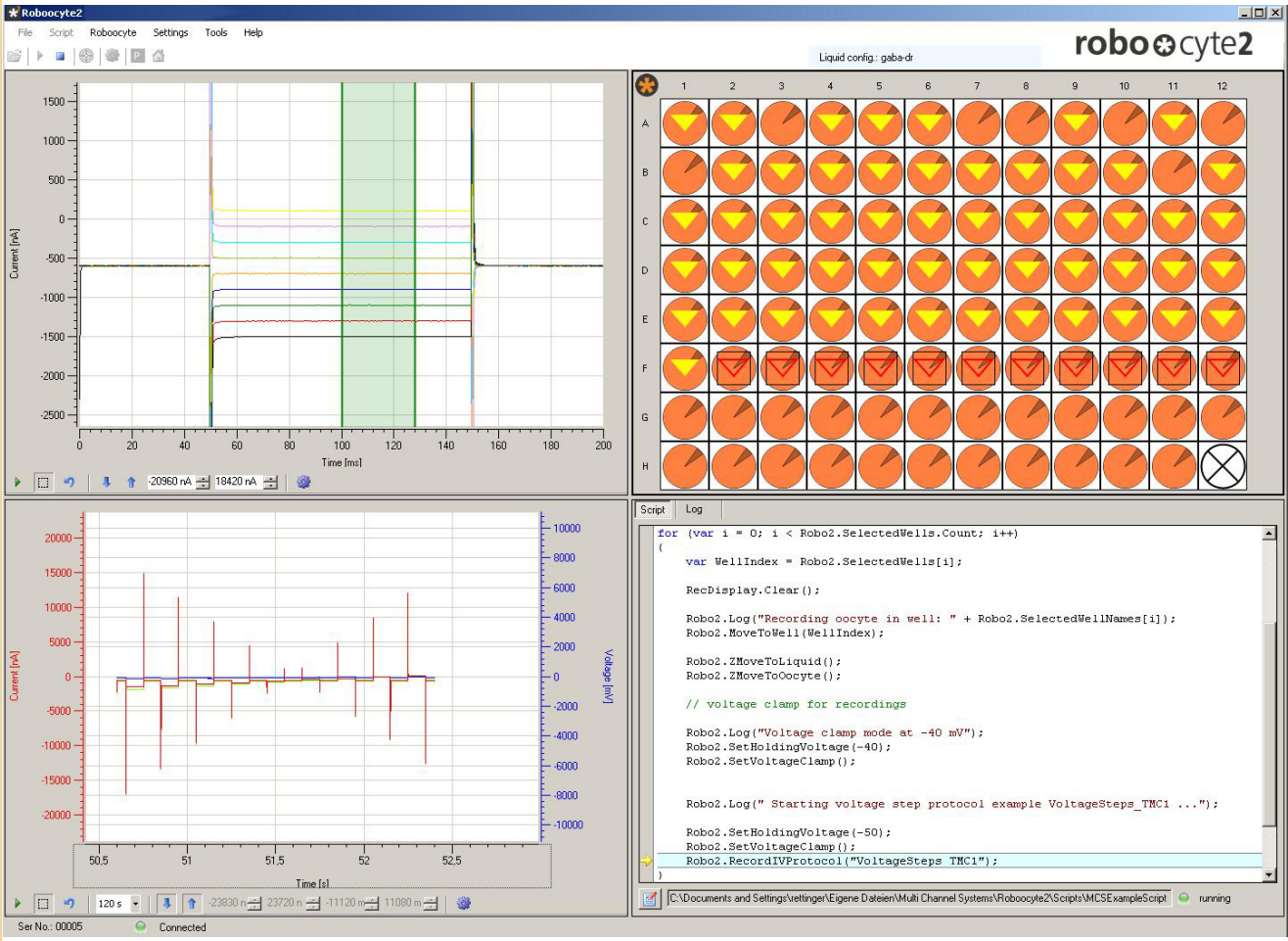
Automated Features

- Two-electrode voltage clamp
- Oocyte impalement
- High throughput compound screening
- Generation of dose-response curves
- Semi-automatic calculation of EC/IC50 values
- Perfusion paradigms
- Response-dependent recording sequences save time and compounds

Advantages

- Full automation and time saving: Once you have loaded a java script, start a recording process by a single mouse click.
- Flexibility and power: Set up several recording protocols for various applications, with full control of all parameters.

Software Details



Easy-to-Use Software Interface

Operate the Roboocyte2, collect and evaluate the data by using the Roboocyte2 software, which has an intuitive graphical user interface and is easy to use.

The well plate view gives a quick overview on the current state of the oocytes. Simply click any virtual wells to select all or specific oocytes for recording. The Roboocyte2 program provides an intuitive manual mode for trying out your experimental settings and for quick tests. The manual operation also provides an opportunity to new Roboocyte2 users of making their first experiences without the need for setting up a detailed protocol first.

The Roboocyte2 software allows a DC offset correction, electrode resistance check, oocyte impalement, compound application, as well as current and voltage clamp both under manual control and in a fully-automated fashion.

Convenient Data Analysis

The recording and control windows display results online throughout testing. Acquisition and real-time analysis are performed automatically from 96 oocytes in a well plate. For example, you can run an automated P/n leak subtraction protocol and extract the extremum of the region of interest. Plots of concentration series and voltage step series are generated automatically. Compound information and results are automatically filed into a Microsoft Access database that offers all the conveniences of an industry proven database for creating reports, managing enormous numbers of compounds, and evaluating results. You can export the data in ASCII format and use your custom evaluation software as well.

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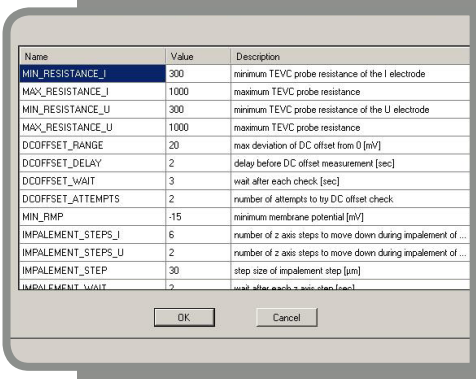
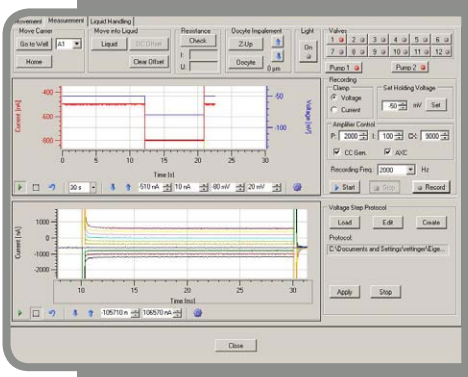
Flexible Recording Protocols

Design your custom parameters and prompt them in a script. You can then flexibly adjust these parameters in a dialog box at the beginning of a recording. For example, you can easily change the holding voltages in a voltage step series from run to run. The use of predefined recording protocols guarantees a quick setup time and a high reproducibility of experiments, preventing handling errors.



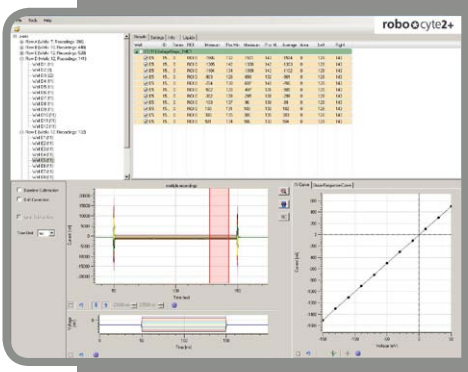
Manual Control Mode

You can test your protocols and perform quick TEVC recordings under manual control. Simply open the "Manual Mode" dialog and enter the well number to move the TEVC probe to the selected oocyte. Control the oocyte impalement by checking the membrane potential. Switch from current to voltage clamp and enter voltage step protocols into the easy-to-use graphical user interface. Apply compounds by clicking the corresponding buttons in manual mode.



Data Analysis with Roboocyte2+ Software

You can define the baseline and a region of interest separately. The settings optimized on the basis of one data plot can be applied to all. Sweeps are grouped, and current-voltage, or concentration curves are plotted automatically. The figure shows a current-voltage curve of a voltage step series recorded from a test model cell.



Scripting Features

Roboocyte2 scripts are written in Java Scripting Language, which allows the full automation of various applications.

You can apply essentially unlimited numbers of compounds to an oocyte that shows correct responses to control stimuli.

You can set up response-dependent recording sequences and control routines with user specified limits for reacting in response to the behavior of an oocyte. The Roboocyte2 skips cells that failed viability testing, thus saving compounds and preventing a further delay of the test sequence. Typical control routines are electrode resistance checks and offset correction, membrane potential and leak current checks, response tests to a reference compound, wash-out checks, and so on.

You can flexibly define the recording sequence for each oocyte separately and integrate control routines of your choice, to save time and be cost effective. You can use functions for often-used routines.

Script templates for typical applications are included, and a competent support team can assist you in writing custom scripts specifically for your applications.

Free Updates and Support

The development of the Roboocyte2 software will always be in progress, because Multi Channel Systems is eager to improve its products and to meet the requirements of the customers. MCS will be glad to hear your suggestions and to add new features. Free software updates will be available to download from the MCS website.

A comprehensive manual, an interactive HTML help, and a friendly and competent support team are provided to aid your progress.

Technical Specifications

Roboocyte2 Robot

- Dimensions: 320 mm x 320 mm x 310 mm (W x D x H)
- Weight: 23.2 kg
- Power supply:
  Voltage range: 100 to 240 VAC
  Frequency: 47 to 63 Hz
- Supply pressure:
  4 to 8 bar @ pressure regulator input
  3.2 bar @ Roboocyte2 input

ClampAmpC

General:

- Newly designed integrated digital TEVC amplifier
- Headstages included
- Operates fully-automatically and computer-controlled
- Active bath clamp with two independent reference electrodes
- Sampling rate: 1 Hz - 20 kHz
- Data resolution: 16 bit
- Recommended electrode resistance range: 100 kΩ to 1 MΩ

Current electrode output:

- Output range: -107 µA to +107 µA
- Effective current resolution: 1 nA
- Compliance voltage range: -100 V to +100 V

Voltage electrode input:

- Input range: -500 mV to +500 mV
- Voltage resolution: 0.125 mV
- Clamp voltage setpoint range: -500 mV to +500 mV
- Clamp voltage setpoint resolution: 1 mV

Amplifier gain settings:

- Proportional gain: 0 - 6700 nA/mV
- Integrator gain: 0 - 8000 1/s
- Typical rise time in voltage clamp mode: <1 ms

Performance and Throughput

- Operates with disposable standard 96-well plates
- Positioning accuracy: 20 µm in x/y and z-direction

Perfusion System

- 12-channel pinch valve Roboflow-System with two peristaltic pumps, adjustable flow rate 0.1 to 10 ml/min via RS232 serial port
- Full integration of an external liquid handler Gilson GX-271 via RS232 serial port
- Either system is fully controlled and automated by the Roboocyte2 software
- Number of compounds is limited only by oocyte performance

Software

- Full automation and control of all devices and features including perfusion via scripting
- Connection to the Roboocyte2 via USB 2.0
- Data export in ASCII file format
- Linkage to Microsoft Access 2010 database (Microsoft Access not included)

Accessories

- Fully-installed Dell computer and 24“ LCD monitor
- Stereo microscope
- Ready to use TEVC probes

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Applications

$GABA_A$ Receptors

Introduction

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system. The GABA type A receptors are pentameric chloride channels assembled from a range of subunit isoforms, which influence the pharmacological properties of the receptor subtype.

$GABA_A$ receptors are targets for many clinically important drugs like anxiolytics, anticonvulsants, anesthetics, sedatives, muscle relaxants, barbiturates, and benzodiazepines, for example valium.

Aim

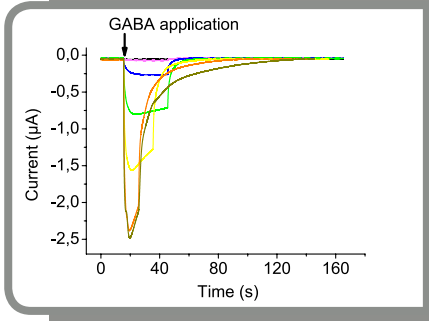
The α_1 and β_2 subunits are expressed for 2-7 days after co-injection of both cDNAs in *Xenopus* oocytes, where they form functional chloride ion channel complexes in the oocyte membrane.

The aim is to analyze the pharmacological properties of this ion channel, for example, the modulation by GABA or the dose-dependent block of GABA induced currents with the Two-Electrode Voltage-Clamp method.

Signals

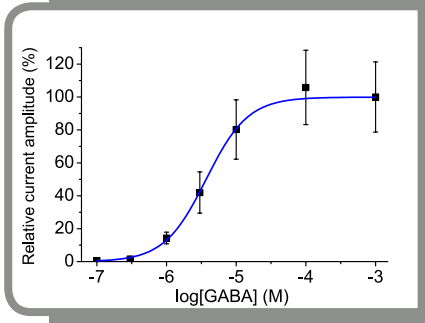
GABA-induced currents were recorded at a holding potential of –60 mV. In general, GABA was applied for 10–30 s to minimize desensitization but to ensure saturating responses also at lower concentrations. The maximum of the GABA-induced current was reached after 2.5 s, and the baseline current was reached again within 20 s after GABA wash-out. A successful recording of GABA-induced currents (with a minimum amplitude of 500 nA) was obtained in about 40 % of the oocytes.

The figure shows an overlay plot of recordings with different concentrations of GABA (0.1 µM, 0.3 µM, 1 µM, 3 µM, 10 µM, 100 µM, 1 mM).



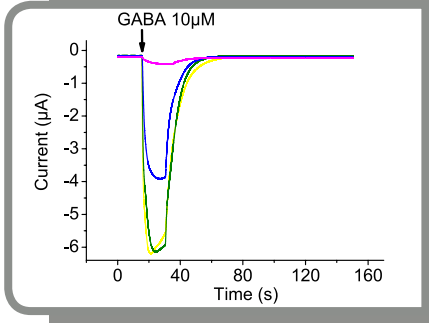
Dose Response Analysis

Currents were normalized to the maximal current obtained with 1 mM GABA. The EC_{50} value for the rat $GABA_AR$ subtype $\alpha_1\beta_2$ determined with automated TEVC was 3.7 µM, which is comparable to EC_{50} values of 5.8 µM or 4.3 µM obtained with conventional TEVC recording.



Inhibition of the $GABA_A$ Receptors by Bicuculline

The figure shows t--he dose-dependent inhibition of GABA-induced currents by bicuculline. Bicuculline was applied for 2 min prior to the application of 10 µM GABA in the continued presence of the drug. All concentrations were tested consecutively and automatically on the same oocyte. Each drug application step was followed by a wash step of 2-5 min prior to the application of the next test concentration.



(Pehl, U., Leisgen, C., Gampe, K. and Günther, E. (2004). „Automated Higher-Throughput Compound Screening on Ion Channel Targets Based on the Xenopus laevis Oocyte Expression System." Assay Dru Dev Technol 2(5): 515-524.)

Applications

hERG Current

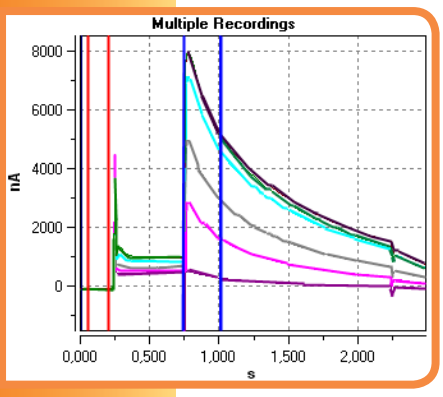
Introduction

Potassium channels critically contribute to cardiac repolarization, that is, to the final phase of the action potential that returns the cell to its resting state. The human ether-a-go-go related gene (hERG) encodes the pore forming subunits of the potassium channel that mediates rapidly activating delayed rectifier K^+ currents (IKr). Drugs that block potassium channels can lead to a prolongation of the action potential.

Aim

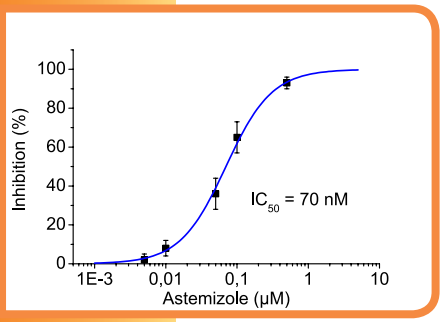
Drug induced Long QT-Syndrome (LQTS) and Torsade de Pointes arrhythmia are a pressing public health issue. Inhibition of hERG is considered a significant risk factor for cardiac safety. In the last few years, a number of drugs have been withdrawn from the market due to adverse cardiac side effects leading to LQTS.

As a consequence, the pharmaceutical industry tends to screen for unwanted side effects of drug candidates on the cardiac action potential already in the earlier drug profiling stage. An automated electrophysiological screening with the Roboocyte can be used to characterize the effects of pharmaceutical compounds on hERG ionic currents.



Recording of hERG Currents

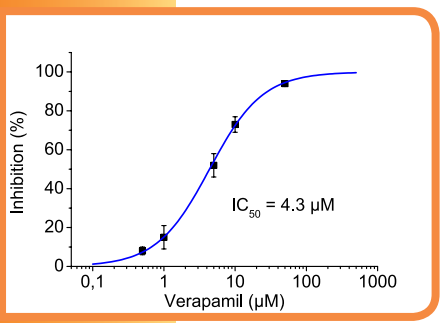
The screenshot from the Roboocyte Analysis window shows an overlay of hERG induced currents. The heterologously expressed hERG channel was activated by a 500 ms depolarizing step to 0 mV from a holding potential of -90 mV, and a steady state current was observed. Since the rate constant for recovery from inactivation is faster than the deactivation rate constant, a step back to -85 mV elicits a large tail current, as there are many channels that have not proceeded from the opened to the closed state. The hERG channels were blocked by increasing concentrations of Astemizole at 5, 10, 50, 100, and 500 nM.



Inhibition of hERG Tail Currents by Astemizole

Astemizole is an antihistamine that provides relief from symptoms of allergies. The drug has been withdrawn from the U.S. market due to cardiac safety problems.

Astemizole blocks the IKr current by inhibition of the hERG K^+ channels. The measured IC_{50} value of 0.069 µM is virtually identical to published data.



Inhibition of hERG Tail Currents by Verapamil

The phenylalkylamine verapamil is used in the treatment of cardiovascular diseases such as angina pectoris, hypertension, and supraventricular tachyarrhythmias.

The IC_{50} value of 4.3 µM measured with the Roboocyte is comparable to published data (3.8 µM) measured with a conventional setup.

(Data kindly provided by IonGate Bioscience GmbH, Frankfurt / Main, Germany, www.iongate.de)

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Applications

Na/K-ATPase Transporter

Introduction

The Na/K-ATPase is a ubiquitous and critically important membrane protein that transports 2 K^+ ions into and 3 Na^+ ions out of the cell against the electrochemical gradient by using the energy of the hydrolysis of 1 ATP molecule per transport cycle.

The transporter serves many functions including creating and maintaining the transmembrane Na^+ and K^+ gradients that contribute to the membrane potential and excitability, driving secondary active transport systems coupled to Na^+ fluxes, and determining a significant fraction of the cellular metabolic rate via ATP hydrolysis. 30-70 % of the cell's ATP is used for this transporter.

Moreover, the Na/K-ATPase is the pharmacological receptor for cardiac glycosides, which are widely used in the treatment of heart failure because of their positive ionotropic effect, and is possibly also the physiological receptor for endogenous ouabain-like compounds.

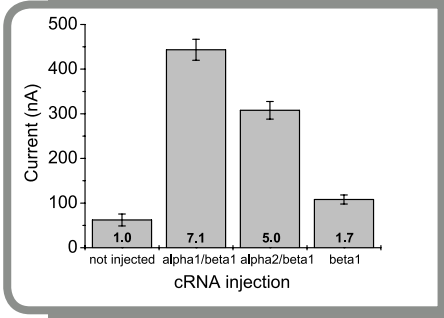
Aim

Four different Na/K-ATPase isozymes were expressed in *Xenopus laevis* oocytes and investigated with the Roboocyte. *Xenopus laevis* oocytes also express an endogenous Na/K-ATPase. To distinguish between the endogenous and the heterogeneous forms, the Na-pump current of injected oocytes was compared with that of non-injected cells.

Compounds can then be tested on oocytes to reveal potential effects on the Na-pump transport activity. In this case, the effect of the inhibitor ouabain on endogenous and human α_2/β_1 Na/K-ATPase was analyzed.

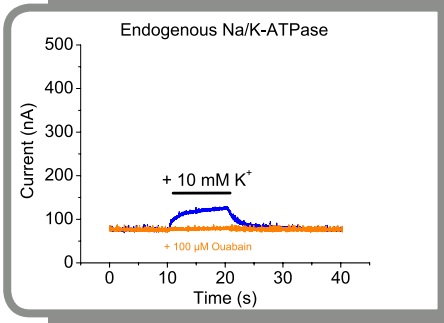
Oocyte Expression

Oocytes injected with cRNA encoding human Na/K-ATPase subunits and preloaded with Na^+ showed pump currents that were 1.7-fold (*Xenopus laevis* α/human β_1 Na/K-ATPase) to 7.1-fold (human α_1/β_1 Na/K-ATPase) higher than those measured in non-injected oocytes (endogenous Xenopus laevis Na/K-ATPase), which is in good agreement with published data. Thus, a discrimination between *Xenopus laevis* and human Na/K-ATPase is possible.



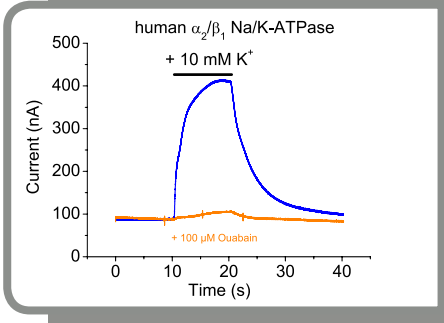
Inhibition of the Endogenous Na/K-ATPase by Ouabain

The effect of the inhibitor ouabain (100 µM, exposure time 2 min) on endogenous Na/K-ATPase is shown. Note, that even the small endogenous currents of only 50 nA can be perfectly resolved with the Roboocyte's digital TEVC amplifier.



Inhibition of a Human Na/K-ATPase by Ouabain

This graph shows the effect of ouabain (100 µM, exposure time 2 min) on the human α_2/β_1 Na/K-ATPase.



(Data kindly provided by IonGate Bioscience GmbH, Frankfurt / Main, Germany, www.iongate.de)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Multi Channel Systems

About MCS

Multi Channel Systems is a rapidly expanding team of highly trained specialists working in a congenial environment. More than two thirds of the employees hold an advanced degree in physics, electronics, informatics, engineering or biology. Combined experience in different technological and scientific fields result in an efficient and creative development of innovative new products.

The excellent communication and strong interaction of the MCS team is the basis for the relationship with you – the customer. This includes not only effective and fast support, but also a close linkage of the product development to the practical, real working conditions of the customers. MCS encourages you to make comments and suggestions, which will be gladly taken up and incorporated in the products, whenever possible. The personal contact with customers and their feedback are very important.

Multi Channel Systems MCS GmbH was founded in 1996 and is based in the Science and Technology Park in Reutlingen in Southwest Germany. The main focus is the development of measuring instruments and equipment for research groups at universities and for the pharmaceutical industry, in the field of electrophysiology. MCS products automate labor-intensive measuring and analyzing processes.

The product line around general non-clinical electrophysiology for measuring electrical activity of excitable cells, *in vitro* and *in vivo*, demonstrates the core competences of Multi Channel Systems. The Microelectrode Array (MEA)-Systems are known worldwide for superior performance and cost-value ratio. The amplifier design is functional and compact. Free software updates reflect the company's philosophy of a flexible evolution of products. MCS always strives to optimize this technology, for example, by adding automated features, real-time feedback circuitry and by miniaturizing components.

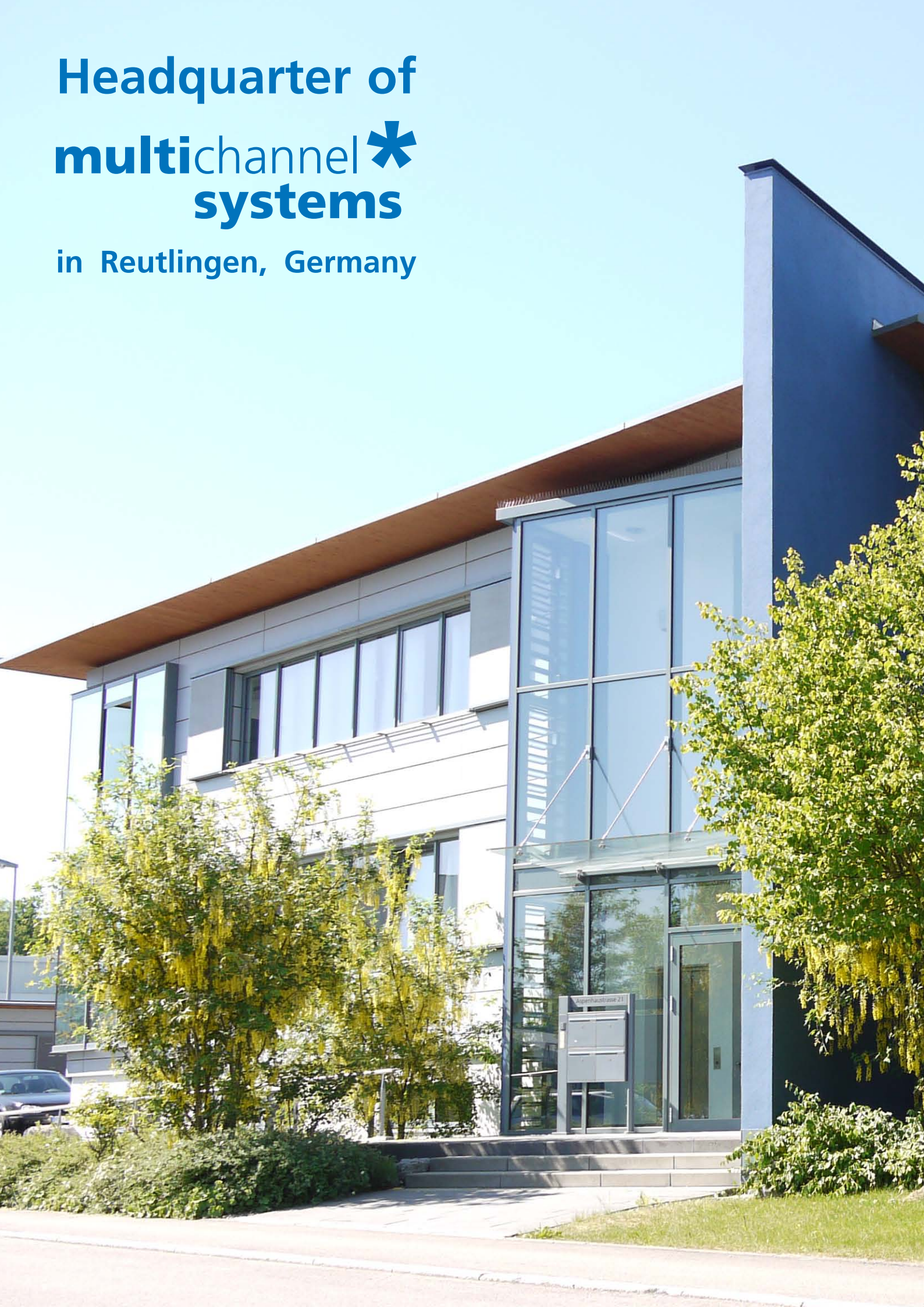
In 2001, Multi Channel Systems (MCS) decided to expand its product line into two-electrode voltage clamp technology with the Roboocyte, the first commercially available fully-automated system for *Xenopus* oocyte screening. Since then, the *Xenopus* research product line has grown and is now complemented with the new Roboocyte2.

The design of the Roboocyte2 also follows our philosophy – to make our products as intelligent, comfortable, and easy to use, and as straight-forward and functional as possible.

Headquarter of

multichannel* systems

in Reutlingen, Germany



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