BIOEXP “FLOW & PATCH”
PATCH-CLAMP APPLICATION

The patch-clamp technique is a gold standard method for investigating ion channel activity at the cellular and molecular level. Using a fine glass micropipette whose tip is covered with a cell membrane patch that forms a high-impedance seal, this technique enables highly sensitive recordings (Fig. 1) of ion currents from both individual channels and entire cells. The BioExp Flow&Patch system is in a league of its own. Its central element, the Flow&Patch chamber (Fig. 2), offers completely new measurement possibilities and additional advantages (see below) compared to other setups, which are often custom-built solutions.

Patch clamp is widely applied in neuroscience, cardiology, and pharmacology, enabling researchers to explore how ion channels regulate signalling, and homeostasis. It also serves to investigate how these processes are affected in diseases such as epilepsy, arrhythmia and cystic fibrosis. This technology is of particular significance within the field of drug research, as it is instrumental in the screening of compounds that modulate the functions of cell membrane channels. Variants such as whole-cell, cell-attached, inside-out, and outside-out configurations provide flexibility to probe intracellular and extracellular channel dynamics, making patch clamp valuable for mechanistic studies and therapeutic discovery (see references).

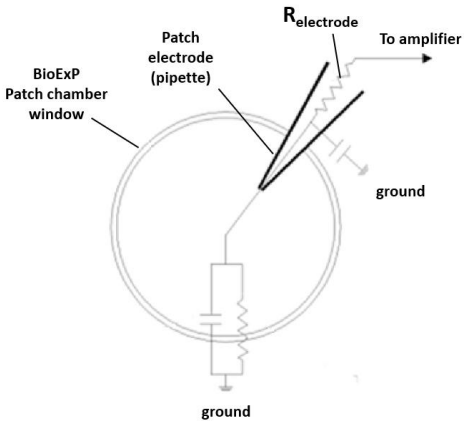

Fig. 1: *Patch clamp set-up principle (above).*

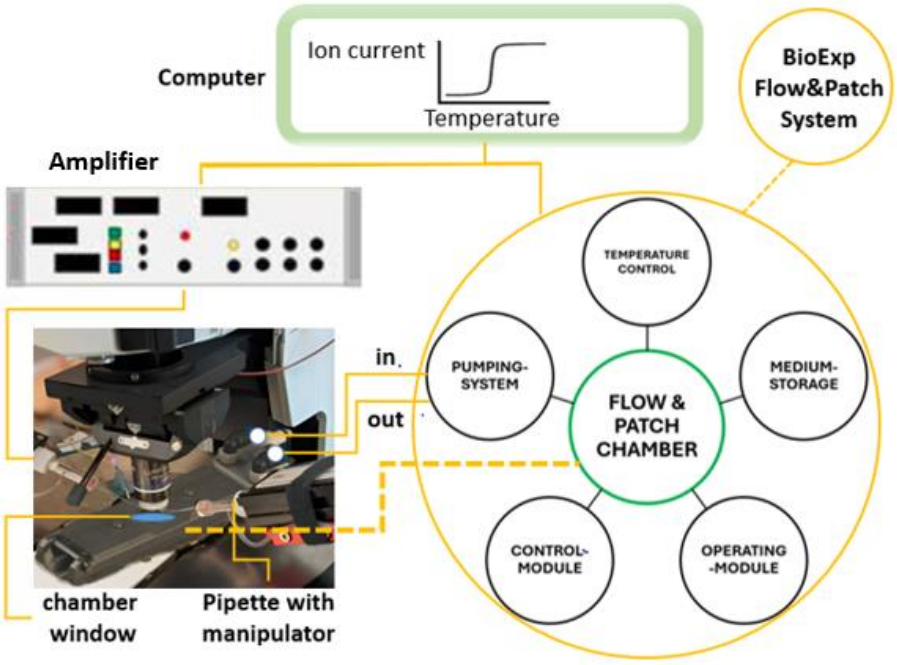

Fig. 2: *Combined patch clamp- and BioExp Flow&Patch systems (right).*

In this application note, we present the innovative BioExp “Flow & Patch” system in combination with regular patch-clamp measurement set-ups. The operation of BioExp's unique, upward-open patch-clamp chamber enables simultaneous high-resolution imaging and patch-clamp recordings under various experimental conditions (temperature, pH value, osmotic pressure, plasma protein content, drug concentrations, etc.) without having to change the membrane patch or the cell currently being examined. The chamber’s design supports the use of a patch pipette at an optimized angle of approximately 45° (±10°), enabling easy access to cell membranes. The system facilitates accurate monitoring of cellular responses to solution changes,

pharmacological agents, and temperature variations, all within a controlled microenvironment. Fully software-controlled solution exchange ensures seamless operation, high reproducibility, and minimal user intervention. The Flow & Patch platform offers a user-friendly, automated alternative to conventional patch-clamp systems, streamlining complex experiments while maintaining precision and flexibility.

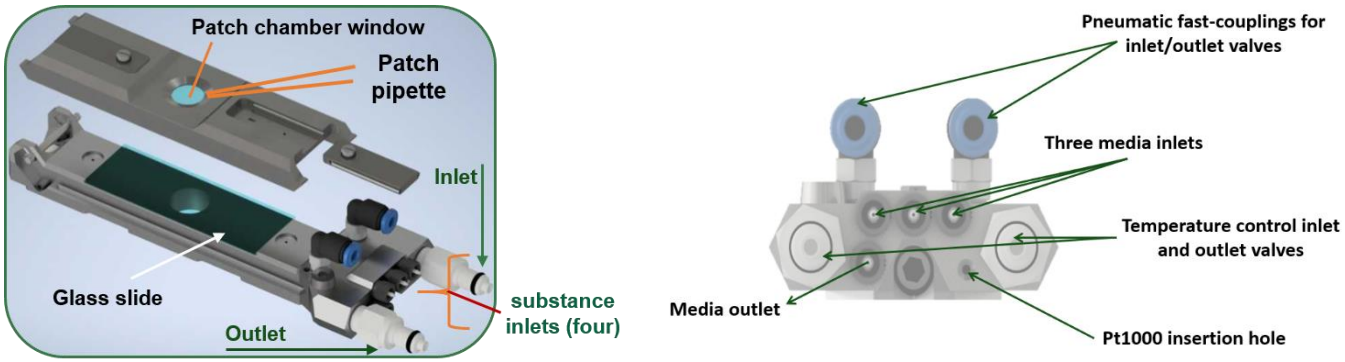

Fig. 3:* Left:** *Schematic of the Flow&Patch chamber;* ***right: *View of the solution feeds for substances and buffer (Pt 1000: temperature sensor*

Keywords: flow-chamber, Patch-clamp, artificial intelligence, pharmacology, shear stress

Advantages of the BioExp "Flow & Patch" system

Technically

- Ergonomic instrument design and fast data collection
- One platform for all: Experiment and evaluation under one (AI) software
- Non-invasive, label-free, time and cost effective
- Compatible with for example Axon molecular devices and software
- Integrated perfusion system and camera
- Temperature adjustable 4-60°C at an accuracy of ± 0.2°C
- Universally compatible with most microscope types.

Experimentally

- Direct agent addition via four inflows, no pre-incubation (physiologically relevant)
- Real-time AI evaluation with user feedback, interactive image review and correction
- Test substance change while the patch is being pipetted allows for:
  - highly accurate dose response data collection and reversibility tests
  - the detection of the drug wash-in and wash-out time constants.
  - Estimation of %-permanent drug action after wash-out

How to use BioExp “Flow & Patch”?

The Flow & Patch system offers a user-friendly, fully integrated flow chamber with four independent substance solution feeds and an upward-opening window optimised for patch-clamp electrophysiology. It supports precise, customisable measurements. Together with its test solution reservoirs, it enables automated and programmable solution exchange and mixing via intuitive software and a multi-channel pump (Fig.2). Unlike conventional patch-clamp systems, which often require complex assembly of separate micromanipulators, perfusion systems, and camera setups, the Flow & Patch, platform features a compact, temperature-controlled flow chamber designed for seamless perfusion of up to four solutions. The render of the Flow & Patch chamber (Fig. 3) illustrates the individual components of the Flow&Patch chamber. It is composed of a top part that possesses an open patch chamber window, enabling convenient access to the cells during experimental procedures. The lower half of the Flow & Patch chamber is equipped with temperature control and four substance solution in- and outlets, as well as the designated surface for microscope slide insertion.

The chamber includes a dedicated angled access window for pipette insertion, ensuring optimal positioning and minimal disturbance during recordings. Figure 2 illustrates the patch-clamp setup combined the BioExP Flow&Patch system. A patch electrode (pipette) is positioned against the chamber window, and its mouth is sealed by a cell membrane patch (Fig. 1). This configuration enables the recording of electrical signals from ion channels within the membrane. The pipette is connected to an amplifier via an electrode with resistance R_electrode, enabling precise measurement of transmembrane currents. The system is fully compatible with for example Axon patch clamp instruments. It includes also all necessary accessories from npi electronic GmbH, such as micromanipulators, amplifiers and a Faraday cage. This makes it a comprehensive, ready-to-use solution. The setup is suitable for measurements with all types of non-adhesive, free-floating cells or permanent monolayer formations of, for example, erythrocytes on a slide (Artmann et al. citation 20, 21). You can cultivate adhesion-dependent cells and then simply pipette them. Cell cultivation on protein-coated slides used in the Flow&Patch chamber (Fig. 3) is carried out according to standard protocols with any cell type in an external incubator. This makes the system a powerful and flexible tool for evaluating the ionic activities of cells and testing drugs, as well as for investigating temperature-dependent cell responses in a controlled, reproducible environment.

The chamber features four independent inlet channels, enabling simultaneous perfusion of up to three test solutions on a single cell monolayer, alongside a dedicated isotonic control buffer. This configuration allows for rapid and seamless switching between solutions during patch-clamp recordings, offering exceptional experimental flexibility. The setup is ideal for studying dynamic changes in membrane conductance, enabling precise comparison of cellular responses under varying conditions.

Test solutions can be customized to include buffers with different pH values, osmotic pressures, or oxygen levels, allowing researchers to mimic physiological or pathological environments. Additionally, the system supports the application of membrane-active agents, such as channel modulators and inhibitors, or chemical modifiers, making it a powerful tool for probing ion channel function, membrane permeability, and cell electrophysiological properties in real time.

Equipped with a high-precision thermostat, the system ensures stable measurements at physiological temperature with a precision of ± 0.2 °C. The variable temperature setting allows users to study temperature profiles (see Artmann et al. citation 21), simulate pathological disorders and investigate their effects cell electrophysiology.

Required instruments for Patch clamp

- PC
- Inverted Microscope
- Microscope Camera
- Microscope Slides
- Cell monolayer*
- Buffer/Test substance
- Micromanipulator
- Faraday cage
- Patch-clamp amplifier
- Flow & Patch chamber
- HiTec Zang BioExp Flow&Patch system for operating the patch clamp chamber (see also Hitec Zang system components and original operating instructions)

*** For experiments involving adherent cell types, we recommend using fibronectin-coated microscope slides seeded with the desired cells. your measurements.**

Commissioning of the combined flow and patch setup

1. Cell layer Preparation

- Adherent cells can be cultured directly on gelatin- or fibronectin-coated glass slides using standard protocols, or transferred onto the measurement slide and allowed to settle and adhere for 10–20 minutes (see references).
- Non-adherent cells and washed red blood cells (RBCs) can be introduced through the measurement window and applied directly onto the measurement slide for further analysis (see references).

2. Flow & Patch Setup

- Install the software **"Patch-Clamp"** and ensure all devices are properly connected.
- Place the flow chamber base (without the top cover) onto the microscope stage.
- Launch the RBC Patch-Clamp software to begin the setup process.
- Position the microscope slide onto the base of the flow chamber.

3. Step-by-Step measurement

i. Heating

The software will run a temperature check. If the internal system temperature is at 37±0.2°C, next step can be started. User is free to skip the heating step if using temperature as a trial parameter was intended.

ii. Position Camera

Ensure that the appropriate connection for docking the camera to the microscope is available (BRESSER MikroCam SP 5.0 microscope camera with C-mount). Attach the camera to the

microscope. If necessary, adjust the camera slightly (adjust the screws forwards or backwards) to ensure horizontal alignment with the flow chamber (can be tested with a short flushing process).

iii. **Place slide inside the chamber**
Open the flow chamber and insert the slides in the desired orientation. Before closing the flow chamber, ensure that the seal is positioned correctly. Place the flow chamber back in the holder.

iv. **Fill supply ducts**
User can utilize this step to ensure the contents of all four medium reservoirs, and will then use the pumps manually to fill the active channels and displace any air bubbles.

v. **Reference Image**
Take a microscopic image of a selected area in the RBC monolayer. This area will serve as a reference for cell counting and must remain unchanged throughout the measurement.

vi. **Start the Patch-Clamp Measurement**

Manual pump adjustment is available during the entirety of the measurement time. All steps are recorded onto the measurement log with a time stamp for easier control of the procedure.

Measurement evaluation

Measurement evaluation is done with the platform provided by npi electronic GmbH.

Ensure confidence in the “Flow & Patch” platform from the Start

Before putting your **“Flow & Patch”** platform to work, a performance validation offers peace of mind by confirming the system is functioning as it should. This simple initial check helps verify proper operation, highlights any setup issues, and lets users get comfortable with the equipment. A quick trial run can uncover potential problems early, ensuring accurate results and smooth performance in every experiment that follows.

Unlock additional applications with Flow & Patch

The system offers additional features and application options that can be tailored to specific research requirements. The AI-based evaluation platform is continuously being developed and can be further customized or tailored at the customer's request. The same experimental setup can also be extended to analyse RBC osmotic fragility, RBC elongation index, RBC-EC adhesion, and RBC shape simply by activating dedicated AI features*.
Furthermore, specialized test protocols such as those for investigating sickle cell disease, diabetes and haemolytic anaemia including the identification of RBC subpopulations are available upon inquiry.

If you're interested in expanding the scope of your research, feel free to contact us to explore the full potential of the system.

***Requires higher hardware specifications than the classic Flow & Patch kit. Available upon inquiry.**

Ready to Get Started?

Your device is designed for precision, reliability, and ease of use. With a successful trial run, you're ready to explore its full potential in your research or clinical workflow.

For further assistance, troubleshooting, or advanced applications, our support team is here to help.

Contact us:

https://cellandtissuetech.com

info@cellandtissuetech.com

https://www.hitec-zang.de

Customer Hotline: +49 171 414 7156

References

1. Neher E, Sakmann B. Single-channel currents recorded from membrane of denervated frog muscle fibres. *Nature*. 1976;260(5554):799–802. https://doi.org/10.1038/260799a0
2. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch*. 1981;391(2):85–100. https://doi.org/10.1007/BF00656997
3. Hille B. *Ion Channels of Excitable Membranes*. 3rd ed. Sunderland, MA: Sinauer Associates; 2001.
4. Sakmann B, Neher E, editors. *Single-Channel Recording*. 2nd ed. New York: Springer; 1995.
5. Yip MC, Lagoy RC, Wang J, Rudy B, Losonczy A. Patch-walking: a robotic multi-pipette strategy for synaptic coupling in the mammalian brain. *eLife*. 2024;13:e84438. https://doi.org/10.7554/eLife.84438
6. Holley SM, Nakano S, Jahandideh S, et al. Automated patch clamp technology for drug discovery and cardiac safety: past, present and future. *Br J Pharmacol*. 2021;178(23):4514–32. https://doi.org/10.1111/bph.15573
7. Nichols CG, Lee SC. Mitochondrial patch clamp: electrophysiology of mitochondrial ion channels. *J Gen Physiol*. 2023;155(10):e202213311. https://doi.org/10.1085/jgp.202213311
8. Liu B, Qin F. Patch-clamp combined with fast temperature jumps to study thermal TRP channels. In: *Methods Mol Biol*. 2019;1987:125-41. https://doi.org/10.1007/978-1-4939-9446-5_9
9. Physiol J. Effects of cooling temperatures on electrophysiological properties via thermal K2P channels. *eNeuro*. 2021;8(5):ENEURO.0308-21. https://doi.org/10.1523/ENEURO.0308-21.2021
10. Van Hook MJ, Nawy S, Thoreson WB. Temperature effects on synaptic transmission and neuronal function in the visual thalamus. *PLoS One*. 2020;15(4):e0232451. https://doi.org/10.1371/journal.pone.0232451
11. Kanade PP, Oyunbaatar N, Lee DW. Effects of low temperature on electrophysiology and mechanophysiology of hiPSC-derived cardiomyocytes. *Microsyst Nanoeng*. 2021;9:9. https://doi.org/10.1186/s40486-021-00135-2
12. Tsurudome K, Ohshiro H, Izumi T. Temperature-dependent hERG channel pharmacology under physiological temperature using semi-automated patch clamp. *Sophion Bioscience White Paper*. 2024; — (QPatch study at 37 °C)
13. Polonchuk L, Bernhard F, Pierson JB, et al. Cross-site variability in automated patch clamp assessment of drug effects on human cardiac currents. *Sci Rep*. 2020;10:5627. https://doi.org/10.1038/s41598-020-62344-w
14. Kramer J, Himmel HM, Lindqvist A, et al. Automated patch clamp assessments: drug effects on cardiac ion channels. *Br J Pharmacol*. 2021;178(23):4514–32. https://doi.org/10.1111/bph.15573
15. Heart et al. Temperature-dependent hERG channel pharmacology and kinetics using APC. *Heart*. [year unknown]; — (record hERG at physiological temperature)
16. Long Y, et al. Drug screening and safety evaluation by patch clamp technique. *J Pharmacol Toxicol Methods*. 2012;65(2):116–26.
17. Molecular Devices, LLC 2021. The Axon Guide (Electrophysiology and Biophysics Laboratory Techniques, Fifth Edition) https://www.moleculardevices.com/en/assets/user-guide/dd/cns/axon-guide-to-electrophysiology-and-biophysics-laboratory-techniques
18. npi electronic GmbH, 2023. Operating instructions and system description for the ELC-03XD UNIVERSAL AMPLIFIER for EXTRA & INTRACELLULAR RECORDING, SINGLE CELL STIMULATION and ELECTROCORPORATION
19. HiTec Zang, C&TT. Original operating instructions for Flow & Patch chamber

20. Artmann GM, Weiergräber OH, Damiati S, Firat IS, Artmann AT. The molecular origin of body temperature in homeothermic species. Am J Physiol Regul Integr Comp Physiol. 2025 May 19. https://doi.org/10.1152/ajpregu.00236.2024
21. Artmann GM. Microscopic photometric quantification of stiffness and relaxation time of red blood cells in a flow chamber. Biorheology. 1995 Sep-Oct;32(5):553-70. doi: 10.1016/0006-355X(95)00032-5. PMID: 8541524.
22. Petkova-Kirova P, Murciano N, Iacono G, Jansen J, Simionato G, Qiao M, et al. The Gárdos Channel and Piezo1 Revisited: Comparison between Reticulocytes and Mature Red Blood Cells. *Int J Mol Sci*. 2024;25(3):1416. doi:10.3390/ijms25031416
23. Thakore P, Alvarado MG, Ali S, Mughal A, Pires PW, Yamasaki E, et al. Brain endothelial cell TRPA1 channels initiate neurovascular coupling. *eLife*. 2021;10:e63040. doi:10.7554/eLife.63040
24. de Coulon E, Dellenbach C, Rohr S. Advancing mechanobiology by performing whole-cell patch clamp recording on mechanosensitive cells subjected simultaneously to dynamic stretch events. *Front Bioeng Biotechnol*. 2022;10:1040200. doi:10.3389/fbioe.2022.1040200
25. Belinsky GS, Rich MT, Sirois CL, Short SM, Pedrosa E, Lachman HM, Antic SD. Patch-clamp recordings and calcium imaging followed by single-cell PCR reveal the developmental profile of 13 genes in iPSC-derived human neurons. *Stem Cell Res*. 2014 Jan;12(1):101–118. doi:10.1016/j.scr.2013.09.014
26. Grimm C, Vierock J, Hegemann P, Wietek J. Whole-cell patch-clamp recordings for electrophysiological determination of ion selectivity in channelrhodopsins. *J Vis Exp*. 2017 May 22;(123):55497. doi:10.3791/55497
27. Mergler S, Garreis F, Sahlmüller M, Reinach PS, Paulsen F, Pleyer U. Thermosensitive transient receptor potential channels in human corneal epithelial cells. *J Cell Physiol*. 2011 Jul;226(7):1828–1842. doi:10.1002/jcp.22514