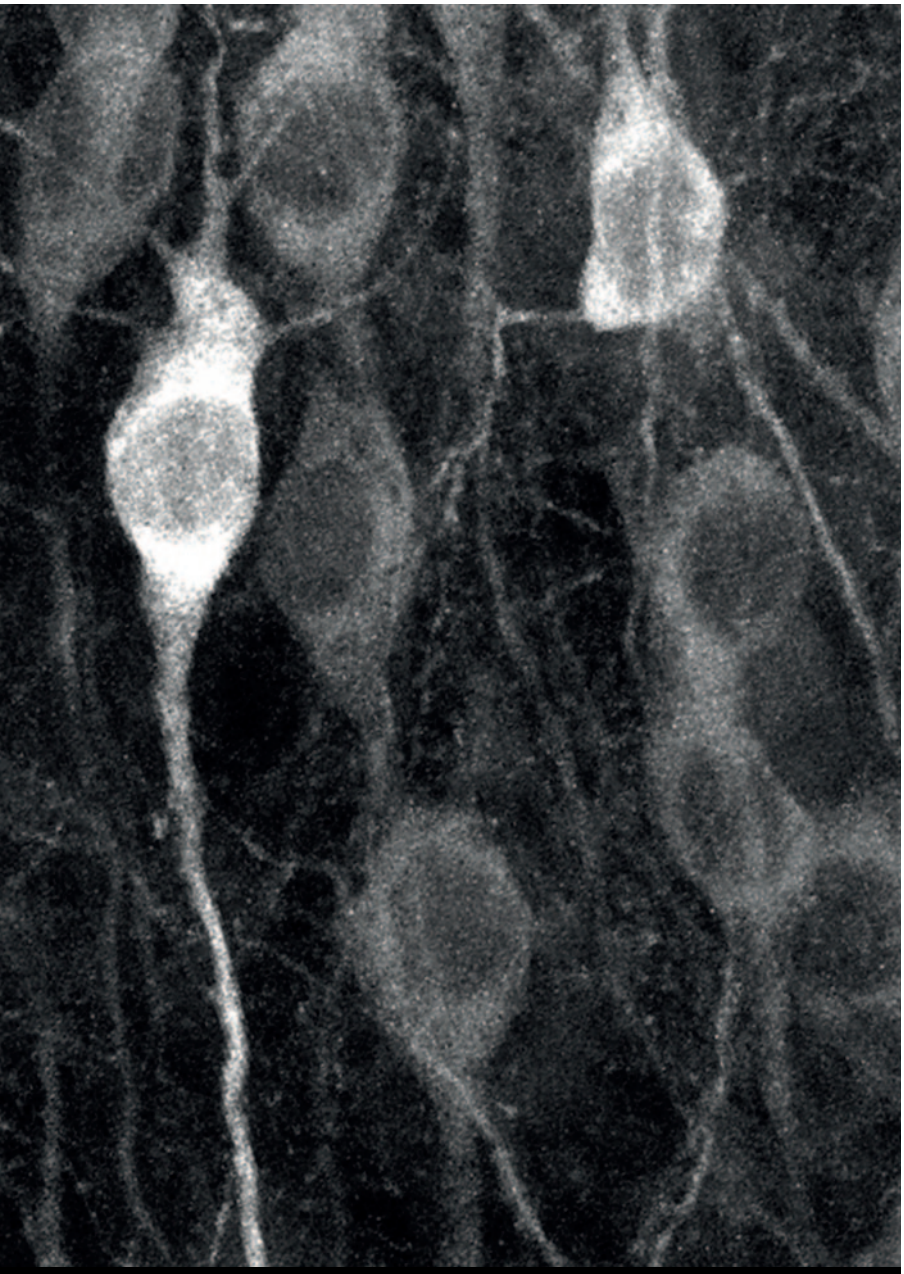
Imaging sodium transients in neurons and astrocytes



The image shows hippocampal CA1 Pyramidal neurons expressing the ATP sensor ATeam1.03YEMK

Key Points

High-quality - FN1 microscope has a range of high-quality optics, enabling visualisation of minute details deep within a specimen with great clarity and contrast

Versatile - FN1 microscope, together with the NIS-Elements software enable work with a wide range of peripheral devices

Intuitive - NIS-Elements software is a professional and easy to use tool with a fully customisable user interface

Cellular sodium homeostasis and signalling in the brain

The maintenance of a low intracellular sodium concentration by the Na^+/K^+-ATPase (NKA) is mandatory for the function of both neurons and astrocytes. Previous studies have suggested that increases in intracellular sodium concentrations ($[Na^+]_i$) are among the first consequences of ATP shortage after a stroke. A failure to control the level of sodium ions in the brain can affect other major ions and neurotransmitters, therefore understanding cellular sodium homeostasis and regulation is critical to the understanding of brain function.

This application note presents data from Dr Gerkau, Heinrich Heine University, Düsseldorf, Germany on how increases in intracellular sodium in astrocytes and neurons drive the reversal of the sodium/calcium exchange (NCX) during peri-infarct depolarisations (PIDs) in stroke. Nikon's stable and reliable FN1 microscope combined with its NIS-Elements software were essential in providing high-quality imaging and analysis of intracellular sodium ions in astrocytes and neurons in living brain tissue.

Imaging of local and global sodium transients

Chemical ion-sensitive fluorescent indicators are widely used for evaluation of intracellular ion concentrations. While today there is a large number of Ca^{2+} indicator dyes, including genetically encoded probes, there are only few recognised chemical sodium indicators available. Dynamic measurement of changes in sodium in astrocytes and neurons with chemical indicator dyes can be achieved with standard wide-field, confocal or multi-photon microscopy. Each of these methods has their special advantages and drawbacks and preference is mainly dependent on the scientific investigation and sample preparation (e.g. cultured cells versus intact tissue).

Multi-photon imaging induces fluorescence emission from the focal plane only which reduces bleaching of out-of-focus structures and enables sodium imaging in subcellular compartments such as dendrites and dendritic spines even in light-scattering tissue preparations. Astrocytes, however, have an extensive 3D structure, and imaging from one focal plane limits analysis to small segments of a cell. Here, wide-field microscopy can be advantageous because it is not confined to the focal plane and therefore, enables fast imaging of a large part of a cell or even the entire cell. Another advantage of wide-field imaging is that it is well-suited for imaging of many cells at a time and thus for detecting activities in cellular networks. Images in this case are obtained as a single frame without the need for scanning, making the technique particularly useful to visualise dynamic processes simultaneously across an object of interest. Importantly, imaging frequencies can be high as well, enabling the measurement of very fast cellular signals.

Wide-field microscopy for imaging sodium transients

Sodium-binding benzofuran-isophthalate (SBFI) is a ratiometric, UV-excitable sodium indicator. Ratiometric imaging enables detection of changes in sodium concentration largely independent from changes in dye concentration, such as induced by cellular movement, bleaching or cellular loss of the dye. Furthermore, *in situ* calibrations enable changes in SBFI fluorescence ratio to be converted into changes in intracellular sodium ($[Na^+]_i$) concentration. When studying astrocytes, preparations can be additionally loaded with the vital dye, sulforhodamine 101 (SR101), which results in their specific labelling and identification.

Nikon's FN1 wild-field microscope is specifically designed for this type of imaging and research, visualising cellular details within *in vitro* specimens with clarity and contrast. In conjunction with an appropriate camera and light source, this system enables high imaging frequencies enabling fast ratiometric imaging. Although a cell's 3D structure is reduced to two dimensions, the wide-field system allows imaging of an entire cell.

In conjunction with this microscope, which is equipped for cellular imaging of ion transients, Nikon's NIS-Elements software serves as a simple interface for photo-documentation which combines powerful image acquisition, visualisation and analysis of data (Figure 1).

Figure 1. Nikon's FN1 microscope equipped with fixed stage, digital camera (Orca Flash 4) and perfusion/microinjection used in conjunction with Nikon's NIS-Elements software.

Case Study

Consequences of Na^+ increases in astrocytes and neurons after a stroke

In core regions of ischemic stroke, disruption of blood flow results in breakdown of ion gradients which can quickly lead to cell death. The surrounding tissues can recover from the effects of the ischemia, but this can be hindered by peri-infarct depolarisations (PIDs) that involve further metabolic stress. Although it has been suggested that some of these changes are driven by concurrent increases in $[Na^+]_i$, there is a lack of information on PID-related increases in $[Na^+]_i$.

Wide-field imaging of Na^+ transients with Nikon's FN1 microscope and NIS-Elements software

Dr Gerkau from the Institute of Neurobiology at Heinrich Heine University Düsseldorf, Germany used multiphoton and wide-field imaging in a rodent stroke model (*in vivo* and *in situ*) to examine changes in Na^+ and Ca^{2+} levels in neurons and astrocytes of the mouse cortex (Gerkau, NJ *et al*, 2018).

In acutely isolated tissue slices, injection of the fluorescent sodium indicator SBFI enabled wide-field imaging of Na^+ transients on Nikon's Eclipse FN1 upright microscope. Astrocytes were labelled with the fluorescent dye, SR101, to allow their identification.

The resulting fluorescence was measured by means of the FN1 microscope (Figure 1). The Nikon's NIS-Elements imaging software was used to control the acquisition and to analyse the outcome. Several important features of the software provided distinct advantages for this work including:

- **Easy incorporation and control of external light sources, such as monochromators or fast LED light and external devices, such as a pressure application device or an electrical stimulator controlled via analogue/TTL signals through NIDAQ**
- **Ability to acquire both the wavelengths and ratio online during the experiment**
- **Ability to measure fluorescence changes over time online and in one window during the experiment and display them as brightness versus time alongside the ratio in real time**
- **Ability to analyse 50 individual regions of interest in one experiment**

To mimic conditions induced following an ischemic stroke *in vivo*, tissue slices were perfused with a saline containing sodium azide (NaN_3, 5 mM) and 2-deoxyglucose (2-DG, 2 mM) which inhibits cellular glycolysis and mitochondrial respiration (a so-called chemical ischemia saline). This manipulation induced changes in SBFI fluorescence that were then converted into changes in $[Na^+]_i$ based on calibration measurements.

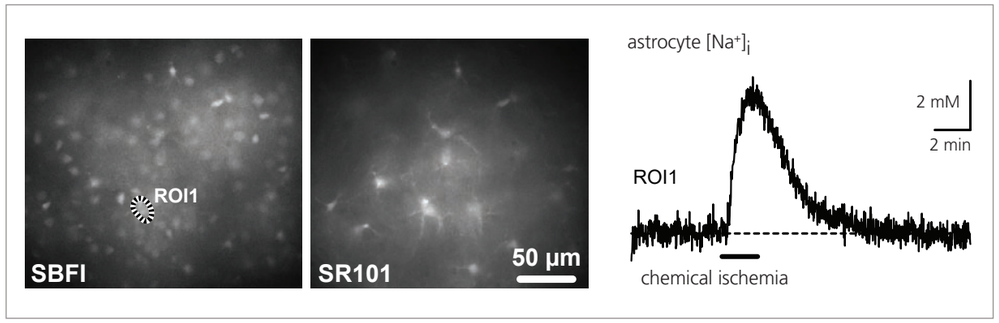


Figure 2. Images of SBFI fluorescence (left) and SR101 fluorescence (centre) of an acute cortical brain slices. Right: changes in $[Na^+]_i$ evoked by a 2-min period of chemical ischemia, induced by perfusion with 5 mM NaN_3 and 2 mM 2-DG in a single astrocyte.

Reversal of sodium/calcium exchanger in neurons and astrocytes may promote recovery from ischemic stroke

After a short period (2 mins) of chemical ischemia, a transient increase in $[Na^+]_i$ was observed in both astrocytes (Figure 2) and neurons. Longer exposure induced a more sustained cellular Na^+ loading that the majority of cells were unable to recover from. Therefore, sodium imaging in this tissue model demonstrated considerable Na^+ loading of neurons and astrocytes accompanying PIDs in ischemic conditions. Further experiments using pharmacological approaches revealed that glutamate transport activity of astrocytes contributed significantly to their sodium loading.

Surprisingly, the measurements also demonstrated that blocking the activity of the plasma membrane sodium/calcium exchanger (NCX) increased Na^+ transients in neurons and astrocytes. On the other hand, Ca^{2+} oscillations evoked by the chemical ischemia were strongly dampened. This suggested that the NCX worked in the reverse mode, mediating Ca^{2+} influx, in addition to driving Na^+ export from both cell types. Na^+ export through reverse NCX may thus promote recovery from ischemic depolarisations. Targeting the NCX exchanger or decreasing cellular $[Na^+]_i$ load could thus represent promising strategies to reduce initial cellular damage resulting from a stroke.

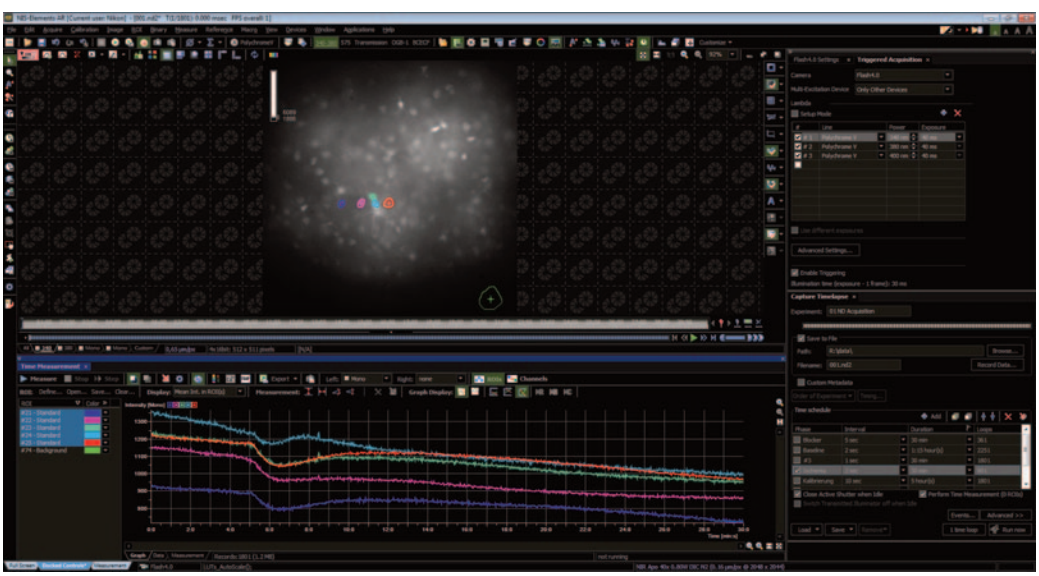


Figure 3. The NIS-Elements software enables high-content analysis of ion transients by quantitative ratiometric imaging across astrocytes and neurons.

Optimal imaging of ion transients in neurons and astrocytes

This work demonstrates that working with Nikon's state-of-the-art FN1 microscope enabled optimal, customised imaging and analysis of changes in intracellular ion concentrations in astrocytes and neurons in living brain tissue. Its stable and reliable function allowed imaging for prolonged time periods. The NIS-Elements software enabled high-content analysis of ion transients by quantitative ratiometric imaging across astrocytes and neurons (Figure 3). By adding a few peripheral features to the microscopy system one can even perform wide-field Fluorescence Lifetime Imaging (FLIM) experiments with the same software.

The combination of Nikon's instruments profoundly benefits this type of research by providing high-quality and versatility to wide-field imaging.

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Dr. Niklas J. Gerkau is a postdoctoral scientist at Heinrich Heine University Düsseldorf, Germany. His current research is focusing on early changes in neuronal and astroglial sodium homeostasis during ischemia in the mouse brain.

References

Gerkau NJ, Rakers C, Durry S, Petzold GC and Rose CR. (2018) Reverse NCX attenuates cellular sodium loading in metabolically compromised cortex. Cerebral Cortex 28: 4264-4280

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