Functional Connectomics: 3D-Reconstruction of active neuronal Networks

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Supported by:

Federal Ministry for Economic Affairs and Energy

on the basis of a decision by the German Bundestag

Report Info:

Report History:
Unpublished Work
Finished: October 23, 2022

Keywords:
Extracellular Recording, connectome, 3D-reconstruction, neural network, extracellular recording

Abstract:

The study of the "wiring diagram of the brain" on different scales is the focus of connectomics, a subfield of neuroscience. However, to understand information processing in a connectome (functional connectomics), it is necessary to visualize the neural network in the brain area of interest in a living organism in real time. This is not yet possible with currently available brain research methods. This technical report demonstrates a new technique for the 3D-reconstruction of the position of active neurons in a neural network. The technique uses extracellular recordings with multichannel microelectrodes (e.g. tetrodes or heptodes) and postprocessing of the recorded data by a special developed software.

1. Introduction

The central goal of brain research is to understand the functionality of the brain. The knowledge gained in this process serves applied and medical research and thus also the society. The information-processing of the brain is based on the activity of a very large number of nerve cells that are interconnected and form so-called neural networks. Neuroscientists use micro-electrodes to obtain information from such neural networks. The main experimental technique used here is electrophysiological multi-microelectrode recording. Micro-electrode manipulators are used to position as many microelectrodes as possible (each of them as thin as a human hair), independently from each other, at different depths of the brain. The greater the number of microelectrodes used, the greater the gain in spatial information.

Understanding the information processing in the three-dimensional neural networks of the brain can lead to the development of neuroprostheses (e.g. visual prostheses for blind people or gait prostheses for people with walking disabilities) or new forms of therapies for neurodegenerative diseases such as Parkinson's or Alzheimer's disease.

The basic investigations are performed on animal models or tissue samples using fiber microelectrodes that are positioned with micrometer precision near the neurons to be recorded by means of a multi-electrode manipulator.

If a neuron becomes active, this leads to a short, measurable spatio-temporal change in the electric field, the so-called “action potential” or "spike". These signals have voltage amplitudes of about 50 - 100 µV. However, with a microelectrode, one can only record electrical signals from the nerve cells locally. To understand the cooperation of several nerve cells in a neural network, it is necessary to know which nerve cell is active at which time and how the flow of information in the network takes place.

The totality of connections in the nervous system of a living being is called a connectome. The study of the "wiring

diagram of the brain" on different scales is the focus of connectomics, a subfield of neuroscience. These studies have so far been conducted with dead organisms and not in real time. However, to understand information processing in a connectome, it is necessary to visualize the neural network on a cellular level in the brain area of interest in a living organism in real time. This is not yet possible with currently available brain research methods.

In this report, we present a new and soon commercially available technique to display the extracellular activity of active neurons in the immediate vicinity of a multichannel recording electrode tip three-dimensionally on a computer monitor screen (see Figure 1 and Figure 2).

Our work is based on the results of Mechler et al. published in 2011 [1, 2], who reconstructed the spatial configuration of sets of simultaneously recorded neurons to demonstrate the potential use of 3D-dipole localization for functional anatomy. The authors investigated the spatial sensitivity of Thomas tetrodes for single unit recording in the brain and they solved the inverse problem of estimating the location and the size of the equivalent dipole model of the spike generator associated with each neuron. Later in 2013 Lee at al. [3] presented the first experimental validation of electrical source localization and intensity characterization using Thomas tetrodes. The results presented by the authors showed, that this method is a good candidate for source identification in both acute and chronic electrophysiological experiments, allowing much needed insight to be gained on neural migration patterns and size-related neuronal functionality.

Motivated by this work of leading neurophysiologists, we at Thomas RECORDING have developed the results presented in this report as part of a research project supported by the German Ministry of Economics (Grant number: EP190669).

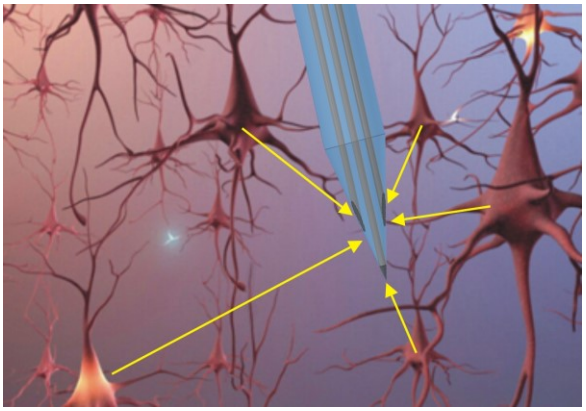

Figure 1: The image shows the tip of a Thomas RECORDING heptode in a neuronal network. The neurons located in proximity of the heptode tip contribute to the extracellularly recorded signal. This is a superposition signal that can be separated into the signal contributions of the individual neurons using appropriate software algorithms.

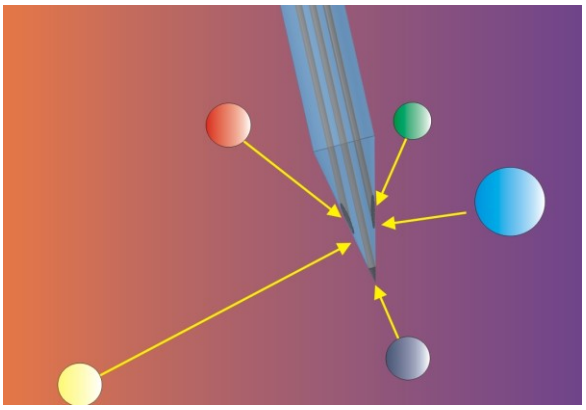

Figure 2: Once the signals of the individual neurons have been extracted from the superposition signal (multi-unit activity), the location of the individual neurons can be determined with respect to the position of the heptode tip and the position of the neurons can be displayed in three-dimensional space as point sources as shown in this picture. The basic principle of three-dimensional localization of neurons using 4-channel microelectrodes (tetrodes, Thomas RECORDING) has already been presented by the research group of Professor Jonathan Victor at Cornell University in New York [1, 2].

2. Materials and Methods

2.1 Description of the Method

The most widely used method in neuroscience for recording extracellular activity from neurons is the use of fixed silicon probe arrays. These fixed electrode arrays have the disadvantage that the electrode contacts are arranged equidistantly and can only be moved together. However, since the nerve cells in the brain are not arranged equidistantly, not all contacts of these fixed electrode arrays will be able to record neural signals. Another disadvantage of fixed silicon probe arrays is that the electrode contacts are arranged only on one side of the silicon probe shaft. This allows signal recording only from the spatial direction the electrode contacts are facing. No signals can be measured on the back side of the silicon probe shaft. Thus, one has only a three-dimensional signal recording from brain structures located on one side of the electrode shaft.

The 3D-reconstruction is based on the acquisition of neural signals from multiple perspectives (triangulation) and is therefore performed by our algorithm only for signals recorded by Thomas tetrodes or heptodes.

Thomas tetrodes and heptodes were developed by Uwe Thomas in 1987 (see Figure 3). These electrodes are quartz glass insulated fiber electrodes with 4 (tetrodes) or 7 (heptodes) metal cores made of a platinum (95 %) tungsten (5 %) alloy. The tip shape of the electrodes is conical. Each electrode has a center conductor and three (tetrode) or six (heptode) conductors concentrically arranged around this center conductor. These multiconductor electrodes allow neural signal recordings from all directions of the three-dimensional space.

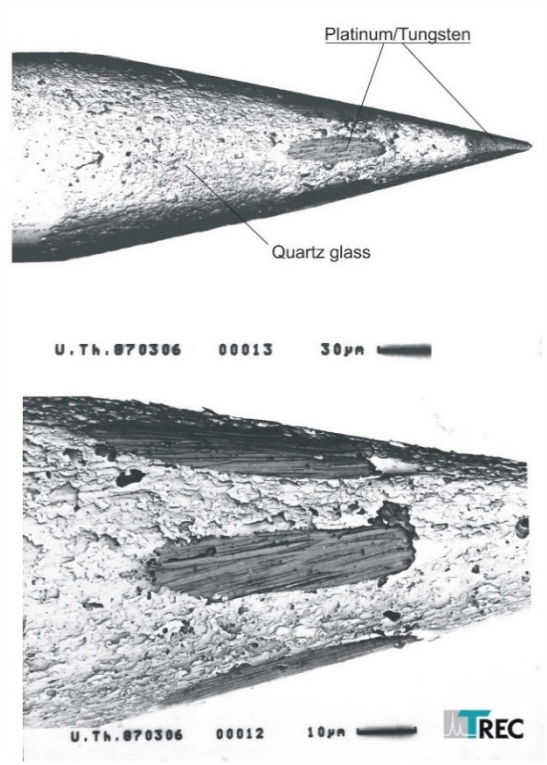

Figure 3: Scanning electron microscope photo of a Thomas heptode tip (Uwe Thomas, March 6, 1987).

In a research project funded by the German ministry of economics between 2014 and 2016 our team at Thomas RECORDING developed a new 3D-heptode based on the original standard heptode technique (grant number: EP140101, see Figure 4).

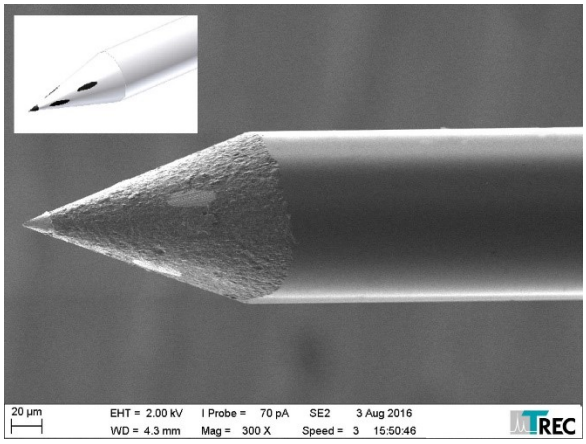

Figure 4: Scanning electron microscope photo of a Thomas 3D-Heptode tip (TREC, 2016). This 3D-heptode has contacts arranged in three levels.

The extracellular action potentials (spikes) captured by the recordings arise from multiple neurons at several distances to the electrode and are generally superimposed by artefacts and noise. After averaging the recorded action potentials (spikes) we obtain signals with high signal-to-noise ratio for each tetrode or heptode channel. This is a prerequisite for accurate 3D-reconstruction of signal source (neuron) position. The determination of the neuron positions is based on the attenuation of the signal amplitude of a spike due to the tissue specific conductivity of the neuronal area between the source (neuron) and the sink (electrode contact) (see Figure 5).

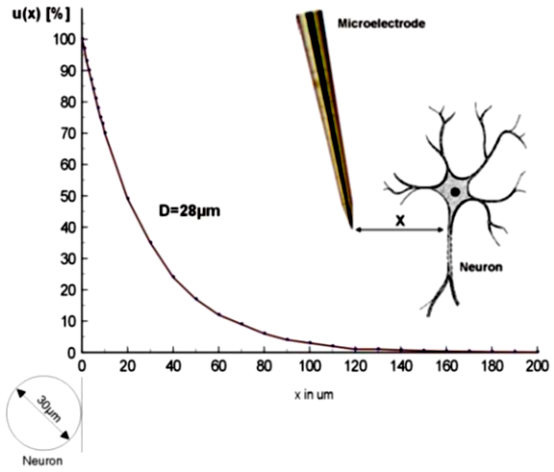

Figure 5: On the cell surface, a recording electrode conducts 100% of the extracellular potential. With increasing distance (x) of the electrode tip from the neuron surface, the signal amplitude u(x) decreases exponentially until it disappears in the neuronal background noise. Due to these biophysical conditions, it is only possible to record extracellular neuronal signals when the distance between the electrode tip and the neuron is comparatively small.

Electrode channels of a heptode or tetrode that are close to the signal source register a less attenuated signal than those that are far away (see Figure 6). A detailed description of the advantages of tetrode signal recording over single electrode recordings was described by Gray et al. 1995 [4].

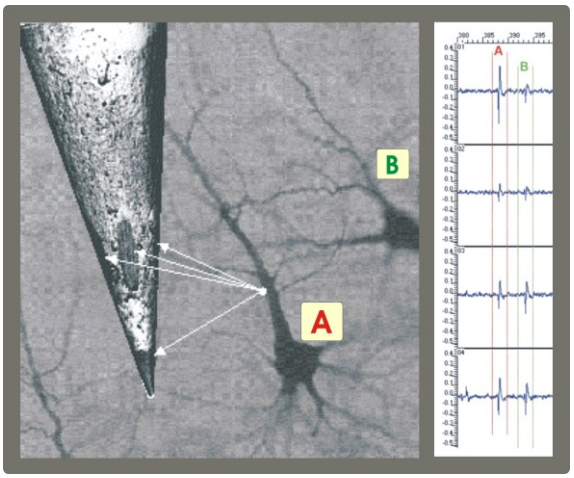

Figure 6: Schematic drawing of a tetrode tip beside neuron A and neuron B (left side), Signals recorded by the tetrode tip (right side). If we assume that neuron A is located closer to the tip of the tetrode than neuron B, then the signals picked up by the tetrode from neuron A have a larger amplitude than the signal picked up from neuron B. The orientation of the signal sink (tetrode contacts) to the signal source (neuron) provides a specific amplitude ratio on the four recording channels of the tetrode that is constant for a source-sink distance. Thus, the signal pattern recorded from neuron A differs from that of neuron B and is characteristic of each neuron in the vicinity of the tetrode tip arrangement (left side of the picture).

For the position determination of the neuronal signal source approximated as electrical point source, we use algorithms of digital signal processing, which we have adapted to the special signal shape and the characteristic properties of neuronal signals. These modified algorithms enable us to determine the direction of reception of individual signal components from a superposition signal of several interference-affected individual signals. In our case, the recorded signal is a superposition of extracellular action potentials of several neurons and noise, which hit the contacts of the recording electrode (Thomas tetrode or heptode) from different directions and are detected by the electrode. The result of the optimization is a vector $\mathbf{r}$ containing the estimated x, y and z positions of the neuron. We chose the mean height on the central

axis between the contacts of the electrode tip as the initial value $\mathbf{r}_0$. Position estimation using our algorithm is performed after each update of the spike sorting process for the averaged signal of each spike cluster, i.e., for the current configuration, whenever ten new spikes are detected. The duration of an optimization process (per neuron position/cluster) is on the order of about 10 ms. The result of this 3D-reconstruction of the network of active neurons in the vicinity of a multicontact electrode (heptode or tetrode) can be seen in the screenshot in Figure 7.

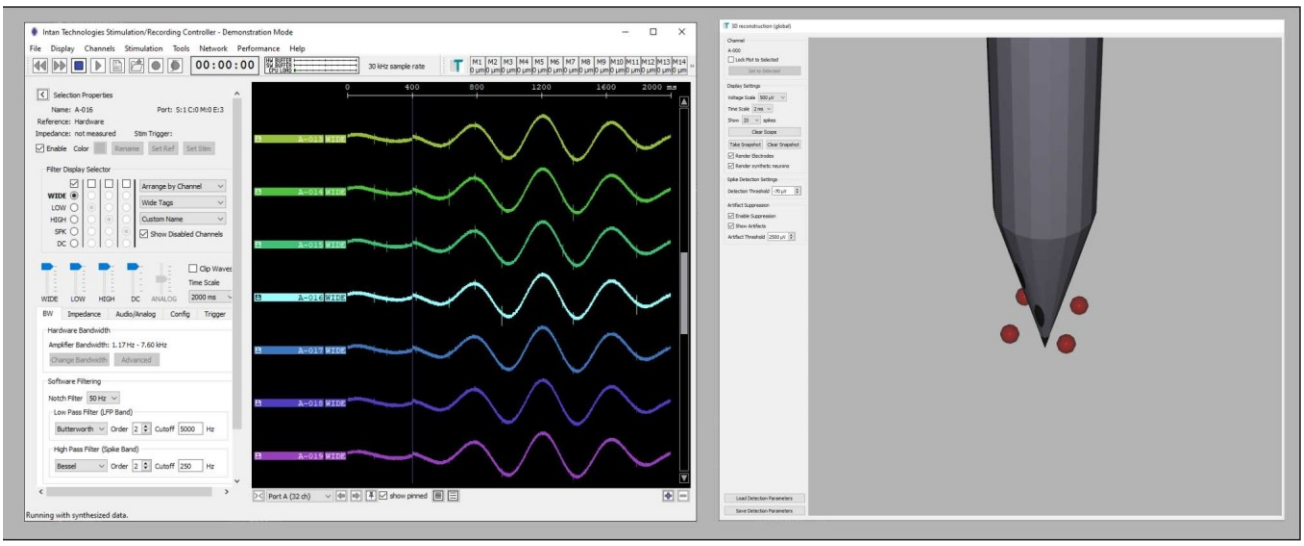

Figure 7: (left) Simulated neuronal signals (spikes) with low-frequency component intended to simulate local field potentials (LFP) and high frequency noise generated to test the functionality of the reconstruction algorithm. Signal amplitudes were calculated using exponential attenuation with increasing distance between signal source (neuron) and signal sink (electrode contact). The signal patterns characteristic for a given source-sink distance were determined for 4 neurons each and stored as superposition signals in the software simulator. (right) The positions of the simulated neurons are shown as red spheres.

Figure 8: If the simulated signals (Figure 7 left) are fed into the 3D reconstruction algorithm, the reconstructed positions of the neurons are obtained. In this figure (left) one can see the electrode tip with the reconstructed neurons. Behind these neurons are the simulated neurons, which are colored red. If one disables the visualization of the simulated neurons and the electrode tip, one sees the pure network of reconstructed neurons around the heptode tip (right image).

2.2 3D-algorithm test setup

We have developed and built a cell simulator that can be used to verify the 3D-neural network reconstruction software under realistic conditions. To find a suitable measurement setup for a cell simulator, one needs to understand the theoretical signal propagation in the extracellular space.

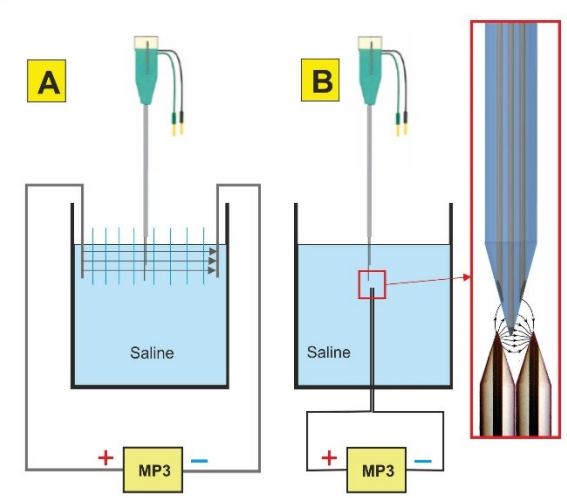

Figure 9: Principle of measurement in the cell simulator
(A) Shows the classical measurement of potential differences in an electric field that forms between two macroelectrodes in physiological saline solution (saline) when a voltage is applied between them. A measuring device detects the potential difference between the recording electrode placed in the center of the electric field and the ground electrode (-). With this lead arrangement, one cannot simulate the conditions in the brain at the cellular level. (B) If the two macroelectrodes from (A) are reduced to microelectrodes (D=80µm) and their tips are brought together to a few micrometers (see B red box), then an arrangement is obtained with which the electrical conditions of an active neuron in the brain can be simulated very well.

Neurons have diameters averaging about 20 - 30 µm, are embedded in an extracellular medium, and act as volume conductors. When the membrane potential differs between two separate regions of such a neuron, there is current flow in the neuron, which is countered by reverse current flow through the extracellular space. Active regions of the membrane are considered as current sinks, and inactive regions as sources. Such extracellular field potentials add linearly and algebraically over the entire volume conductor (principle of electrical superposition). Within the physiological frequency range from 0 to about 5kHz, the inductive and magnetic (wave) effects of the bioelectric signals in the extracellular space can be neglected. This allows a simplified description of the current electrodes as static point sources that can be described according to Ohm's law (for details see [5]). Since the extracellular medium can be considered as an ohmic resistor, a current flow through this resistor generates a voltage drop that can be measured with electrodes. Depending on the location of the neuron where the potential is measured, it has a different amplitude. Furthermore, the amplitude u(x) of an action potential of a neuron measured extracellularly, against a reference electrode far away from the discharge site, decreases exponentially with increasing distance x of the discharge electrode from the neuron (see Figure 5).

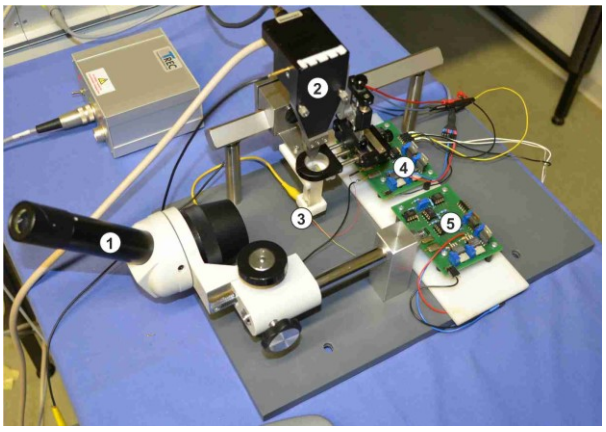

Figure 10: Experimental setup with the cell simulator, consisting of (1) monoscope for positioning the recording microelectrode in the electrolytic trough (3) using a Thomas microelectrode manipulator type “Mini Matrix” (2) and injection of a constant current using a battery-powered, earth ground floating constant current source (4, 5).

We constructed the cell simulator by incorporating a bipolar stimulation electrode assembly as shown in Figure 9 (B) into a glass cuvette (#4 in Figure 10). This bipolar stimulation electrode is connected to a constant current source (#4 and 5 in Figure 10) fed with previously recorded neuronal signals from a battery powered MP3 player. An electric current is formed between the two poles of the stimulation electrode, generating an electric field. A software-controlled microelectrode manipulator (#2 in Figure 10) is used to move a recording electrode with micrometer accuracy near the stimulation electrode array and measure the electric potential. More specifically, the potential difference ($u=\varphi_{electrode} - \varphi_{Ref}$) between the recording electrode and a distant reference electrode is measured. The XYZ manipulator (#2 in Figure 10) and the monoscope (#1 in Figure 10) are used to pre-position the microelectrode manipulator. The experimental setup with the cell simulator is shown in Figure 10.

For the verification of the 3D neural network reconstruction algorithm under conditions as close to reality as possible, the cell simulator was modified. Instead of one bipolar stimulation electrode, two bipolar stimulation electrodes were now used to inject neuronal signals previously recorded in the brain into the electrolytic trough via two independent constant current sources. Signals from two battery powered MP3 players are injected in the electrolytic trough via a Thomas tetrode which was ground flat at the tip (left in Figure 12). Signal were recorded via a Thomas heptode (right in Figure 12). Two independent signal sources (A and B in Figure 12) allow simulation of two separate neurons that are spatially very close to each other. Via the signal recording with the heptode and the 3D-reconstruction from the recorded signals, the position reconstruction of the feed points (position of the contact pairs of the tetrode) should be possible. Figure 12 shows the complete test setup. A detailed description of the components of the test setup is provided in the caption of this figure. The left picture in Figure 11 below shows the positions of the stimulation electrode (tetrode) and the recording electrode (heptode) in the electrolytic trough of the simulator test setup shown in Figure 12. The right image in Figure 11 shows the 3D-reconstruction of the position of the signal source (stimulation electrode, tetrode), represented by three closely spaced spheres beside the recording heptode tip. The position of the signal source (tetrode) relative to the tip of the recording electrode (heptode) could be reconstructed from the recorded neuronal signals (see also C in figure 12).

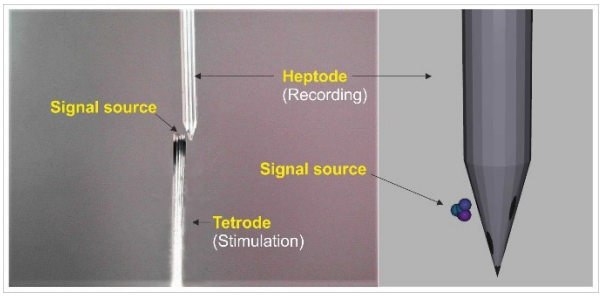

Figure 11: (Left) Signal-injecting tetrode (signal source) and signal-recording heptode. (Right) Signal-recording heptode shown enlarged with some small, differently colored spheres representing the reconstructed position of the signal source. The three differently colored spheres at the same position are resulting from the signal injection of a neuronal signal with signal components from three different neurons. The signal injection was made between two poles of the stimulation electrode (tetrode).

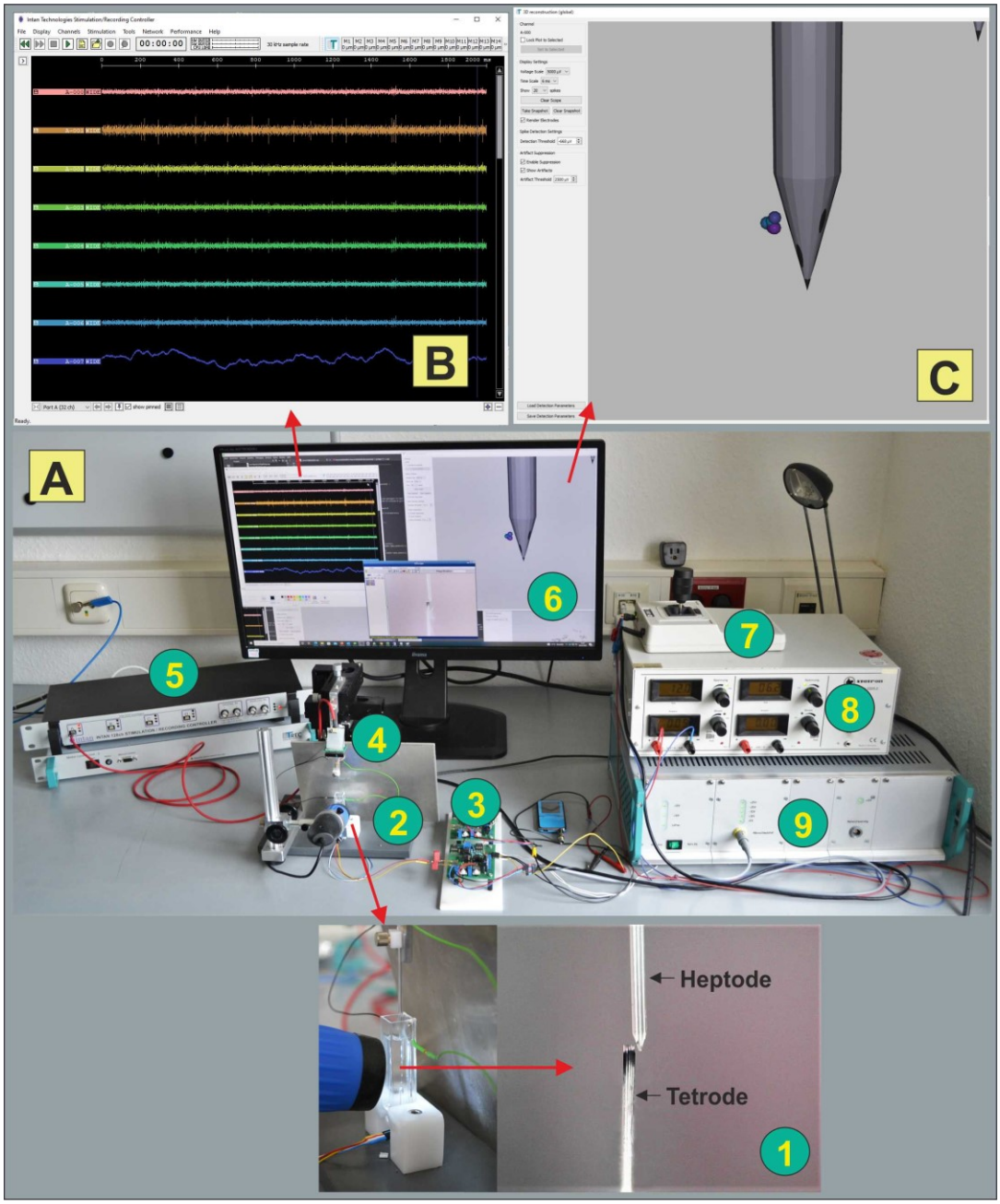

Figure 12: (A2) Simulator setup with electrolytic trough, (A1) recording electrode (heptode) and stimulation electrode (tetrode), (A1) microscope image of the electrolytic trough with heptode and tetrode, (A3) Constant current source with signal generator, (A4) preamplifier for signal recording from the electrolytic trough via a heptode, (A5) data acquisition system, (A6) computer monitor for signal display and evaluation, (A7) motor control for positioning of the recording electrode (heptode) in the electrolytic trough, (A8) laboratory power supply, (A9) mains-isolated power supply for the constant current source. (B) Signals recorded with the heptode, (C) Result of the 3D-reconstruction. The reconstructed signal source (tetrode) is on the left side of the heptode tip (see C), which is the same position as in the electrolytic trough (see A1).

The following figures show individual process steps of the 3D reconstruction, starting with the simulated original signal "recorded" by the heptode from four simulated neurons and ending with the positions of the four neurons reconstructed in three-dimensional space.

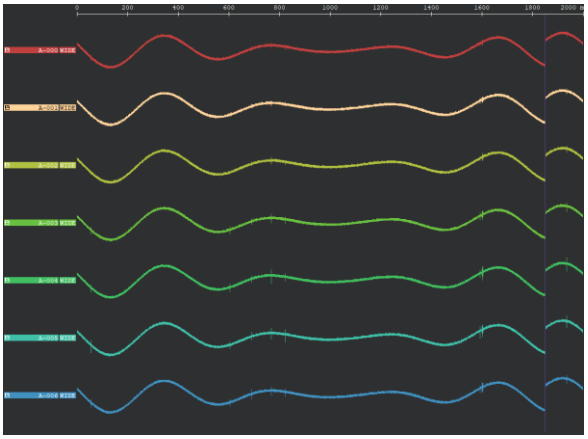

Figure 13: The recorded signal of the heptode. The visible spikes originate from four neurons simulated as point sources

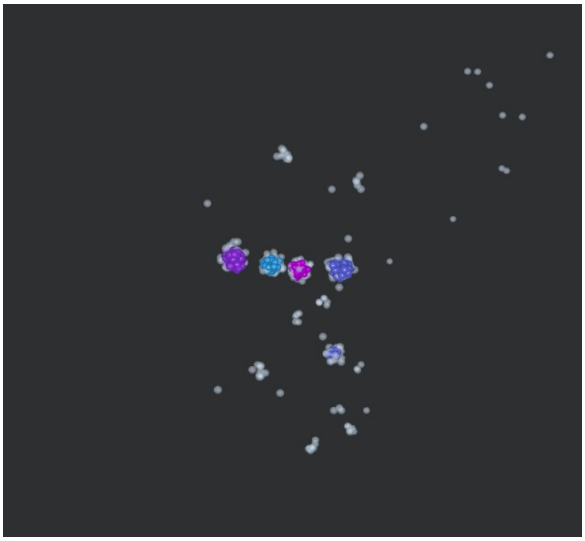

Figure 14: Representation of the Principal Component space. The first three principal components of each spike candidate identified by the AI are represented in the three dimensions. Signals whose principal components are close to each other in space are likely to come from one and the same signal source. Signals are assigned to individual clusters (colored spheres) using DBSCAN (density-based clustering)

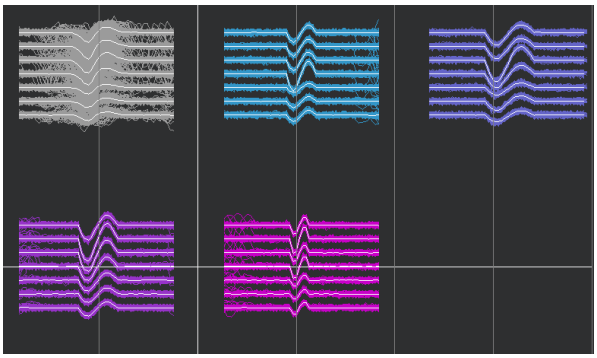

Figure 15: The signals from the clusters from "Heptode PCA clustering.PNG" are superposed for each channel and displayed in the corresponding colors. The white lines represent the averaged signals of each channel. These averages are also used for 3D reconstruction in further processing.

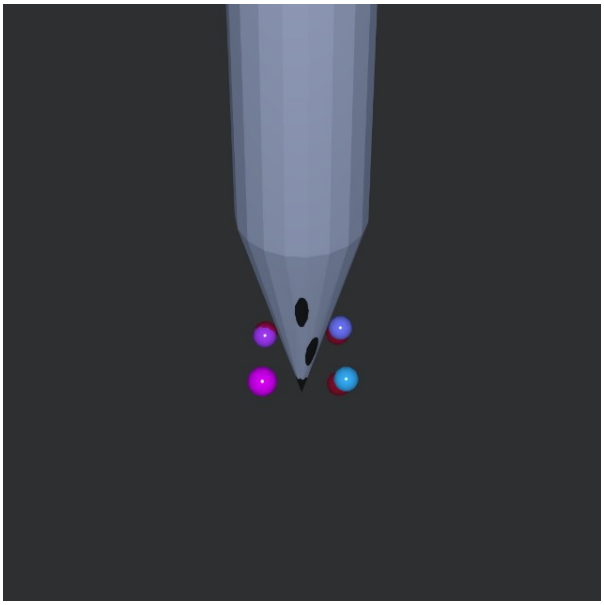

Figure 16: Shown in space are the heptodes, the true positions of the four simulated neurons (red) and the reconstructed positions (colored, corresponding to the other images).

Figure 17: Only the reconstructed neuron positions are displayed.

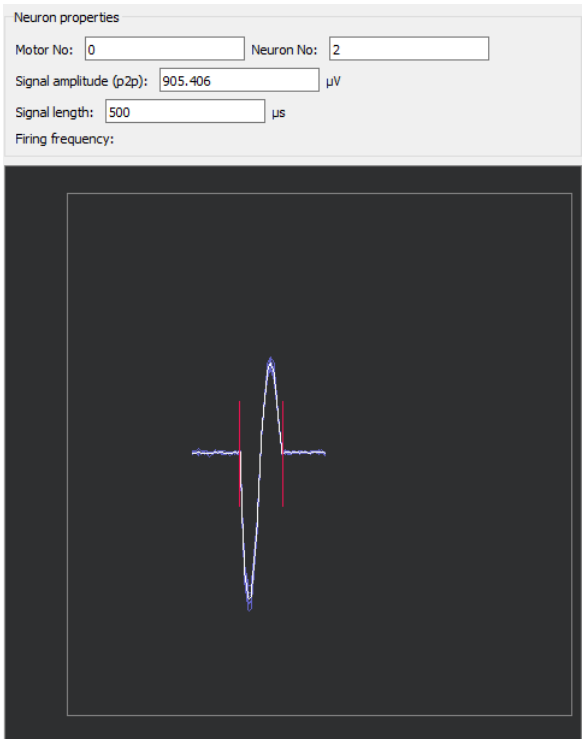

Figure 18: By means of the estimated origin (x,y,z) of a signal, the signal at the neuron is reconstructed via the attenuation law and thus some measured quantities are determined (amplitude, duration, firing frequency).

We are currently working on further optimizing the software. We plan to validate the software with in vivo experiments in rodent and NHP in the next months.

3. Grants

This work was supported by the Federal Ministry of Economic Affairs and Energy (BMWI, Germany) grant numbers EP140101 and EP190669 both awarded to Thomas RECORDING GmbH, Germany.

4. References

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