Control and data acquisition software for high-density CMOS-based microprobe arrays implementing electronic depth control

Karsten Seidl1,*, Tom Torfs2, Patrick A. De Mazière3, Gert Van Dijck3, Richard Csercsa4,6, Balazs Dombovari4, Yohanes Nurcahyo1, Hernando Ramirez1, Marc M. Van Hulle3, Guy A. Orban3, Oliver Paul1, Istvan Ulbert4,5, Herc Neves2 and Patrick Ruther4

1 Department of Microsystems Engineering (IMTEK), Microsystems Materials Laboratory, University of Freiburg, Freiburg, Germany
2 Interuniversity Microelectronics Center (IMEC), Leuven, Belgium
3 Laboratorium voor Neuro- en Psychofysiologie, K.U. Leuven, Leuven, Belgium
4 Institute for Psychology, Hungarian Academy of Sciences, Budapest, Hungary
5 Peter Pazmany Catholic University, Budapest, Hungary
6 Corresponding author: Karsten Seidl, Department of Microsystems Engineering, Georges-Koehler-Allee 103, D-79110 Freiburg, Germany
Phone: +49-761-203-7202
Fax: +49-761-203-7192
E-mail: seidl@imtek.de

Abstract

This paper presents the NeuroSelect software for managing the electronic depth control of cerebral CMOS-based microprobes for extracellular in vivo recordings. These microprobes contain up to 500 electronically switchable electrodes which can be appropriately selected with regard to specific neuron locations in the course of a recording experiment. NeuroSelect makes it possible to scan the electrodes electronically and to (re)select those electrodes of best signal quality resulting in a closed-loop design of a neural acquisition system. The signal quality is calculated by the relative power of the spikes compared with the background noise. The spikes are detected by an adaptive threshold using a robust estimator of the standard deviation. Electrodes can be selected in a manual or semi-automatic mode based on the signal quality. This electronic depth control constitutes a significant improvement for multielectrode probes, given that so far the only alternative has been the fine positioning by mechanical probe translation. In addition to managing communication with the hardware controller of the probe array, the software also controls acquisition, processing, display and storage of the neural signals for further analysis.

Keywords: electronic depth control; neural recording; silicon microprobes; three-dimensional (3D) probe arrays

Introduction

Recordings of single neuron activity with high spatial resolution are required for a basic understanding of neural processes [11, 27]. This goal is currently achieved only by silicon based MEMS arrays realized as one-dimensional (1D) [21, 24], two-dimensional (2D) [14, 19, 24, 36] or three-dimensional (3D) electrode configurations [1, 7, 27, 28]. Despite recent advances, these microelectrode arrays comprise a comparably small number of electrodes which contradicts the need of neuroscientists to record the activity from small neurons, e.g., the visual cortex where somas are approximately 20–50 μm in size, and to record from different layers within the cortex with a total thickness of several millimeters.

The current number of electrodes per shaft is limited by space constraints, i.e., minimal line width and spacing of internal leads defined by lithography. Therefore, to increase the number of electrodes, the integration of electronics on the probe shaft itself is mandatory. So far, electronics have only been integrated in the larger connecting areas of the probes [4, 27] or on a separate chip attached to the backbone of the probe arrays [13] due to the fact that specific process technologies applied to realize these silicon-based probes are often incompatible with the integration of electronics on the probe shaft [7, 27].

Aside from the large number of electrodes required for high-density recordings, long-term recording is often inhibited by micromotions of the recording probe in neural tissue which can increase the distance between a recording electrode and the neuron of interest. Because close proximity between electrode and neuron is mandatory for the discrimination of single action potentials, these micromotions can hinder the quality of recorded signals. In contrast to single wire electrodes, manual adjustment of the probe position is not an option in the case of multielectrode arrays as the position of only one electrode can be optimized each time. In addition, signal quality can degrade over time due to apoptosis, tissue drift, relaxation, inflammation and reactive gliosis, among other reasons. Hence, there is a need for (re)selecting the electrodes which are richest in information about the firing activities of the cells.

Recently, a new concept of electronic depth control with CMOS-based hardware has been presented [25, 33]. Restrictions of existing systems, namely the limited number of electrodes on microfabricated probes and the required mechanical position control to compensate for micromotions, are circumvented. The slender, silicon based probe shafts contain integrated CMOS circuitry which allows the mini-
mization of the number of connecting lines and at the same time enables the selection of a subset of recording sites from an unprecedented number of electrodes [33].

The task of finding high quality signals in neural recording typically depends on the operator’s intuition and subjective assessment with the aid of oscilloscopes and loudspeakers [9, 22]. In the case of multielectrode arrays with a large number of recording channels as proposed by Seidl et al. [33], a manual selection is tedious and rather impracticable [5]. By contrast, (semi-)automatic selection is required that aims to identify the best recording channels out of a set of electrodes. This selection is based upon a signal quality metric, i.e., the signal-to-noise ratio (SNR). Adding signal processing functionality to rate the signal quality and to (re)select the best electrodes results in a closed-loop design of a neural acquisition system. The NeuroSelect software presented in this paper has been developed to (i) control the innovative CMOS-based neural probes with electronic depth control, (ii) process the recorded neural signals, (iii) select appropriate electrodes with optimized signal quality based on the data processing, and (iv) display and (v) store neural information.

Materials and methods
CMOS-based neural probe array
As described in detail elsewhere [33], the CMOS-based neural probes developed within the NeuroProbes project [30] comprise slender probe shafts (Figure 1) with integrated circuitry making it possible to switch electronically between different electrodes. The neural probes are realized in a post-CMOS compatible process combining deep reactive ion etching with sputter deposition of electrode arrays having an electrode pitch of 40 μm with an exposed electrode diameter of 20 μm. In addition to the probes with a shaft-length of 4 mm introduced by Seidl et al. [33], systems in a different CMOS technology with shaft lengths of 2 and 8 mm are currently fabricated. The probes are being implemented in 1D-, 2D- and 3D-arrays.

The integrated circuitry of the CMOS-based probes comprises a switch matrix to select simultaneously eight recording sites per shaft from a total of $N$ electrodes. With the selection, each electrode can be switched to one of two possible lines out of the total eight analog output lines A1 to A8, as illustrated in Figure 2. The switching matrix itself contains a shift register formed by a chain of D-type flip-flops which allows the serial programming of the switches using two control lines (data input, DIN, and clock, CLK) in combination with two lines for power supply (VSS and VDD) [33]. Details on the integrated circuitry are given elsewhere [33].

As shown in Figure 2, the electrode selection code is sent from the host computer via a controller to the microprobe. Neural signals are acquired, displayed and saved. By adding signal processing functionality to rate the signal quality (described in the next section) and to (re)select the best electrodes results in a closed-loop design of a neural acquisition system.

Depth control software NeuroSelect
The software NeuroSelect was developed to control the CMOS-based multielectrode arrays and record neural signals based on the selection of best performing electrodes. It provides the following features:

- graphical user interface;
- communication with the hardware controller of multielectrode probes and data acquisition;
- data processing of recorded neural signals to extract the signal quality metric;

Figure 1 Schematic of the active probe shaft with a length of 4 mm comprising rows of electrodes with a pitch of 40 μm.

Figure 2 Closed-loop system design. (A) Electrode selection is transferred from the host computer via a controller to the microprobe. Neural signals are recorded and visualized. Based on the computed signal quality the electrodes are (re)selected. Selection is achieved via a shift register comprising flip-flops (FF): (B) elementary cell of switch matrix, (C) single node with switch and FF.
electrode selection based on the quality metric either in a manual or semi-automatic mode by selecting best performing electrodes;
- data display, and data storage.

These features are described in detail below.

**Graphical user interface** The NeuroSelect software provides a Graphical User Interface (GUI) that integrates the components for data acquisition (DAQ), signal processing and communication with the hardware controllers. As shown in Figure 3, the GUI is split into different windows that can be resized individually. The upper left pane is used to control the data acquisition from the DAQ card PCIe 6259 from National Instruments as detailed later and to define the file name for the recorded signals. The left center pane is used to configure the plot settings, i.e., data scaling and selection of electrode signals to be displayed. The neural signals acquired from the DAQ card are visualized in the main window in the center. The bottom pane gives feedback about the current status of the software as well as the hardware. The right side window shows the control panel for electrode selection and settings. When maximizing the right pane (Figure 4), one is able to select the electrodes of the different probe types in a manual or semi-automatic mode as described in the section on electrode selection.

**Data acquisition** As each CMOS-based neural probe shaft provides eight analog output channels, 32 and 128 signals are provided by the 4-comb and 4×4 platform arrays, respectively. These signals are pre-amplified using a custom-made CMOS amplifier and fed to the data acquisition cards (PCIe 6259 DAQ, National Instruments) with 16-bit resolution, sampling rate of 31.25 kHz per channel and up to 32 analog inputs. Four of these cards are required to acquire all signals from a full 4×4 platform; one is sufficient for a single comb. The auxiliary digital inputs (available on a connector to the interface electronics) can be treated like an additional analog channel. Data are acquired in blocks of 4096 samples, which correspond to the block size in the stored file.

The software part for the data acquisition is included in a multithreaded way to avoid a bottleneck within NeuroSelect. Moreover, the processing of the GUI is slower and less important than the data acquisition process. There is a double buffer object that is used for visualizing the acquired data on the screen (Figure 3, central window). Owing to applied multithreading, gapless data acquisition is guaranteed during the computations.

**Data processing: the on-line spike detection algorithm** The computation of the signal quality of each channel depends on the detection of spikes in those channels.

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**Figure 3** GUI overview of software for electronic depth control with subwindows for electrode selection, control for data acquisition, data visualization, plot settings and status information.
The literature offers many spike detection algorithms of which some require no user intervention, i.e., unsupervised algorithms. These are based on amplitude detection [31], nonlinear energy detection [17, 20, 35] and wavelet based detection [8, 18, 23]. Other algorithms are supervised, i.e., they require user intervention, such as window discrimination [37], principal components analysis [2] and matched filtering [6]. We have implemented unsupervised algorithms that allow for the detection of spikes without the need for user interaction. In our case, it is preferable to avoid user interaction as much as possible given that the fine-tuning of every single parameter in a multielectrode setup such as the CMOS-based NeuroProbes array is a far too tedious job for the experimenter. Furthermore, the algorithm has to be efficient in terms of processing time to minimize the scan duration of the electrode array which can consist of up to 8192 electrodes in the case of the 3D-array with 4 × 4 8-mm long probe shafts. In a study by Obeid and Wolf [26], spike detection algorithms have been compared taking into account their accuracy and their computational cost. It was found that taking the absolute value of the neural signal before applying a threshold in combination with a refractory period is just as effective for spike detection as more elaborate energy-based schemes. Therefore, we investigated both the simpler detectors, i.e., thresholding (abbreviated as TH) and thresholding in combination with the absolute value operator (abbreviated as STH), and the more elaborate energy-based detector, i.e., nonlinear energy detector (abbreviated as NEO). We compare the performances of the TH, STH and NEO detectors for depth control in the results section.

**Adaptive-threshold spike detection** The TH and STH methods are basic unsupervised spike detection algorithms still used today, e.g., in [29]. In the case of both methods, the noise level of the signal is estimated by means of the median absolute deviation from zero which is known as a robust estimator of the standard deviation of the background noise [23]. The estimated noise level is defined as:

$$\hat{\sigma}_{\text{noise,TH}} = \frac{\text{median}(s(t)-\bar{s}(t))}{0.6745},$$

where the time dependent signal and its average are written as $s(t)$ and $\bar{s}(t)$, respectively. The hat symbol stresses the fact that the result is an estimate. Generally, the noise level can be estimated from $\hat{\sigma}_{\text{noise,TH}} = C \cdot \text{median}(s(t)-\bar{s}(t))$, where the scale factor $C$ depends on the signal distribution. Assuming a Gaussian signal distribution, $C$ can be calculated by the 75%-quantile of the standard normal distribution to $C = (\Phi^{-1}(0.75))^4 = (0.6745)^4$ where $\Phi^{-1}$ is the inverse standard normal cumulative distribution function [15].

This noise estimation of Eq. (1) is repeated for every consecutive time window of 50 ms, which leads to a noise envelope adapted to the nature of each signal. For the TH method, a spike occurs at position $t$ when the signal value $s(t)$ exceeds
the noise envelope $\hat{\sigma}_{\text{noise},\text{TH}}$ by a factor of $K$, i.e., $s(t) > K\hat{\sigma}_{\text{noise},\text{TH}}$. The STH method works similarly, but takes into account that spikes can have negative deflections as well, as shown in Figure 5 based on the simulated data discussed in the results section. The corresponding inequality is then obtained by taking the absolute value of the left hand side of the previous inequality: $|s(t)| > K\hat{\sigma}_{\text{noise},\text{TH}}$. The factor $K$ is set to an appropriate value, typically 3 or 4 [29].

**Adaptive nonlinear energy detector** The NEO detector is a further extension of the STH method in that a nonlinear operator is applied to the original signal $s(t)$ before the aforementioned STH equation is applied. The nonlinear operator transforms the original signal into the new one, called $s_{\text{NEO}}(t)$, which is defined as:

$$s_{\text{NEO}}(t) = s(t)^2 - s(t - \delta) s(t + \delta),$$

with 0.25 ms as a common value for $\delta$. It has been shown that the NEO detector is not only sensitive to larger amplitudes compared with both the TH and STH method, but also to higher frequencies [17, 20]. These higher frequencies and higher amplitudes occur precisely during spike windows. The noise level $\hat{\sigma}_{\text{noise},\text{NEO}}$ can be computed in the same way as for TH and STH using the signal $s_{\text{NEO}}(t)$ instead of $s(t)$ in Eq. (1). But, $K$ now needs to be set at higher values, e.g., $K_{\text{NEO}}$ can be set to 9 ($= 3^2$) or 16 ($= 4^2$). Hence, quadratic values of the previous thresholds are used owing to the squaring of the $s(t)$ in the $s_{\text{NEO}}(t)$ computation.

**SNR metric for spike quality assessment per channel**

Using the detected spike windows and the signal as input, the SNR is computed as the relative power of the spikes compared with the background noise. Once the spikes are detected, the root mean square (RMS) is computed for $N$ samples within the window indicated by rectangles in Figure 5B around a spike stamp (circles in Figure 5B). The RMS values of all detected spike windows are then averaged with the width of the windows recommended to be taken over the whole time interval of a spike [22]. As an example, the width of the time windows in Figure 5B was set to $t_w = 1$ ms. The SNR value is calculated as:

$$\text{SNR}_{\text{dB}} = 20\log_{10} \frac{1}{N} \sum_{n=1}^{N} \text{RMS}(\text{spike}_n(t)) / \hat{\sigma}_{\text{noise}}(t),$$

where $\text{RMS}(\text{spike}_n(t))$ is a quality measure for the individual spike and denotes the root mean square of spike $n$ as shown in Figure 5B. The ensemble average over all $N$ spikes detected in a particular channel is indicated by

$$\frac{1}{N} \sum_{n=1}^{N} \text{RMS}(\text{spike}_n(t)).$$

$\hat{\sigma}_{\text{noise}}$ is the estimate of the noise standard deviation calculated by the RMS of all mean centered values outside the spike windows which are the pure noise segments.

**Electrode selection** Prior to any data acquisition, the user has the choice between different probe types. Probes with different shaft lengths (2 mm, 4 mm and 8 mm) and different probe configurations (single-shaft probes, probe combs with four probe shafts or 3D probe arrays comprising $4 \times 4$ probe shafts) are currently fabricated within the NeuroProbes project and can be selected within the NeuroSelect software. Furthermore, the gain factor for the CMOS-based pre-amplifier is set in this window of the GUI. The probe type selection is followed by the electrode selection mode as schematically shown in Figure 6. The user is offered the choice between a manual electrode selection mode and a semi-automatic mode for electrode selection. In the manual mode, the user is not supported by data analysis and corresponding calculation and sorting of the quality metric as in the semi-automatic mode. In the future, this might be extended.
Figure 6 Program flowchart of manual and semi-automatic electrode selection.

with a fully automatic mode which selects the electrodes with the best signal quality using predefined selection constraints, i.e., single electrode or tetrode configurations and spacing of the electrodes.

Manual electrode selection mode Up to eight electrodes per shaft can be simultaneously selected by mouse clicking on the electrode symbols of a single-shaft probe, a single comb or the combs of a 3D 4×4 array as shown in Figure 4. In the case of the whole array, the user can select within 2D planes comprising four probe shafts each. These planes are oriented parallel or orthogonal to the probe combs of the 3D array. The selected and deselected electrodes are color-coded as indicated in Figure 3. Furthermore, the user has the possibility to either choose single electrodes or tetrodes, i.e., a set of 2×2 neighboring electrodes. Each electrode can be switched to one among two output lines as illustrated in Figure 2B. Thus, only a certain selection of single electrodes is possible. The rules stemming from this interconnection scheme are implemented in the software so that non-selectable electrodes are disabled for user selection and are again color-coded. The corresponding node structure of the electrode selection matrix can be visualized if required, as shown in the inset of Figure 4.

Semi-automatic electrode selection mode As shown in the program flow chart in Figure 6, the semi-automatic scan comprises a sequential selection of blocks of eight electrodes per shaft, signals of which are recorded during a user defined time and saved in the European data format (EDF). The signal quality metric, i.e., the SNR, is calculated for each block of electrodes as described above. The recording time is adjustable and requires a stabilization time of 30 s for the applied CMOS pre-amplifiers. To reduce the total recording time, the user can constrain the scanning area by selecting a coarse section of the probe in which the electrodes are to be scanned. The results of the SNR computations are visualized by color-coding the electrodes. It is possible to adjust the lower limit of the selected SNR values in the GUI. Based on the information of the signal quality, the user finally selects the appropriate electrodes manually.

Data visualization and storage Visualization is done using the LabWindows/CVI library from National Instruments. An example of signals generated using a function generator is given in Figure 3. Visualization of the recorded data as a superimposed view of single spikes with the computed SNR and firing rate, both in real time, is currently under development. The recorded signals are saved under the user-defined file name in the EDF specified in Ref. [12]. The EDF writing function consists of a code to create the standard headers defining the number of channels, etc., and the code to write the sample data to the file. The latter works very efficiently together with the data acquisition card, because the National Instruments DAQ library is used to gather exactly one block of 4096 samples for all 33 channels (32 analog channels + 1 digital channel) per data acquisition card directly into memory. Because this memory buffer already has the right data structure, it is then written to the EDF file by a single write call. During acquisition, the header file contains “–1” (unknown) in the number of data records. This header field is then filled in with the correct number after termination of the acquisition [16] (Ref. [16] was not cited in the original paper. Please cite accordingly in the text).

Programming environment The software NeuroSelect has been written in C++ and uses the multiplatform framework wxWidgets distributed under free software license for GUI implementation [34]. It has been developed for Windows, but can be ported to Linux, MacOS or other platform environments. Visual Studio is used as integrated development environment (IDE) and compiler, but the Microsoft foundation classes (MFC) are not used. The design of the graphical user interface was developed using the Dialog-Blocks editor from Anthemion [3]. The signal analysis package is developed in C/C++ and uses the OpenMP library in the parallel-processing version. This allows all available processors (and cores) of the computer to be used to process multiple signals in parallel. The source code to control the data acquisition cards (PCIe 6259 DAQ, National Instruments) and to visualize the neural data has been gen-
Results

The performance of the SNR algorithms, i.e., TH, STH and NEO spike detection, used to extract an appropriate signal quality metric has been validated. Simulated extracellular recording from an intrinsically bursting layer-5 tufted pyramidal cell are used as signal with known spike properties. The simulations are based on a neuron model generating a ground-truth, i.e., without noise, spike train with a firing rate of approximately 40 spikes/s. The model is implemented in the Genesis script-language by Dyhrfjeld-Johnsen et al. [12] using morphological reconstruction by Schubert et al. [32]. A typical ground-truth spike train is shown in Figure 5A. We added to this simulated signal different levels of noise each generated by 100 realizations of white Gaussian noise of different standard deviation $\sigma_{\text{noise,sim}}$ (Figure 5B). The increasing simulated noise levels can be interpreted as an increasing distance between the recording electrode and the axon hillock.

Figures 7–9 show extracted SNRs of the different algorithms calculated using Eq. (3) as a function of the normalized noise level $\sigma_{\text{noise,norm}} = \sigma_{\text{noise,sim}} / s_{\text{max}}$, where $s_{\text{max}}$ denotes the maximum amplitude of the simulated spike train $s_{\text{sim}}(t)$. The step size of the normalized noise level used for SNR calculations is set to $\sigma_{\text{noise,norm}} = 0.01$. The error bars in Figures 7–9 indicate the standard deviation on the computed SNRs for the 100 different white Gaussian noise realizations. In each graph, three cases are plotted, namely, for $K=3$, $3.5$ and $4$ in the case of TH and STH methods and for $K=3^2$, $3.5^2$ and $4^2$ in the case of the NEO method. The gray diamonds indicate for each curve the maximum normalized noise level ($\sigma_{\text{noise,max}} / s_{\text{max}}$) where a difference of 0.01 in noise level can still be discerned in the SNR calculation. This was statistically confirmed using a t-test with a significance level of $p=0.05$.

As a result derived from Figures 7–9, the SNR metric is a monotonic function of the underlying noise level for the TH, STH and NEO methods, i.e., the SNR decreases with increasing noise level. This monotonic dependency is desired, because a ranking of the electrodes is done based on the computed SNR metric.
As further extracted from Figures 7–9, the maximum normalized noise level \( \sigma_{\text{noise, max}} / \sigma_{\text{max}} \) is 0.29 in the case of the TH method with \( K_{\text{TH}} = 3.5 \), 0.36 in the case of the STH method with \( K_{\text{STH}} = 3 \), and 0.32 in the case of the NEO detector with \( K_{\text{NEO}} = 3^2 \). Hence, the STH detector can distinguish in a statistically significant manner between different signal qualities at higher noise levels compared with the TH method and the NEO method. Moreover, it requires lower computational costs compared with the NEO method.

**Discussion**

The implemented computation of the SNR computation is based on the STH detector and shows the intended descending monotonicty with increasing noise level. The application of the simpler STH detector in electronic depth control shows a similar performance as the more sophisticated NEO detector. However, the STH detector is easy to implement and fast in performance. This is important for the real-time analysis of multielectrode arrays. If the analysis is implemented with electronic circuitry, the STH detector has the additional advantage that, given its simplicity, it consumes far less power. The conclusion that the STH detector is as effective as the NEO detector was also reached when both detectors were applied for spike detection [26]. Clearly, alternative ways to compute the SNR can be envisaged. Instead of the root mean square of spikes \( \text{RMS}(\text{spike}_w(t)) \) in Eq. (3), the peak-to-peak amplitudes (PTP) of single spikes as in Ref. [17] could be used. However, we preferred the RMS measure, as the PTP amplitudes are expected to be more sensitive to outliers in the data.

The above described STH algorithm is embedded in the NeuroSelect software which enables the experimenter to visualize the recorded signals, spikes, as well as the calculated signal quality metric such as the SNR value per electrode and their relative ordering with regard to each other. The software also manages the recording of the signals, the storage of these signals into EDF files, the execution of the SNR calculation algorithms, and the control of the electronic circuitry to record from the electrodes as selected manually by the user or by a semi-automatic method. Electronic depth control will provide neuroscientists with an advanced method to both adjust individual electrodes of a CMOS-based multielectrode array with regard to specific neuron locations initially after the probe insertion and to track the neurons during long-term recordings.

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