Appendix A. Machine drawings of microdrive components

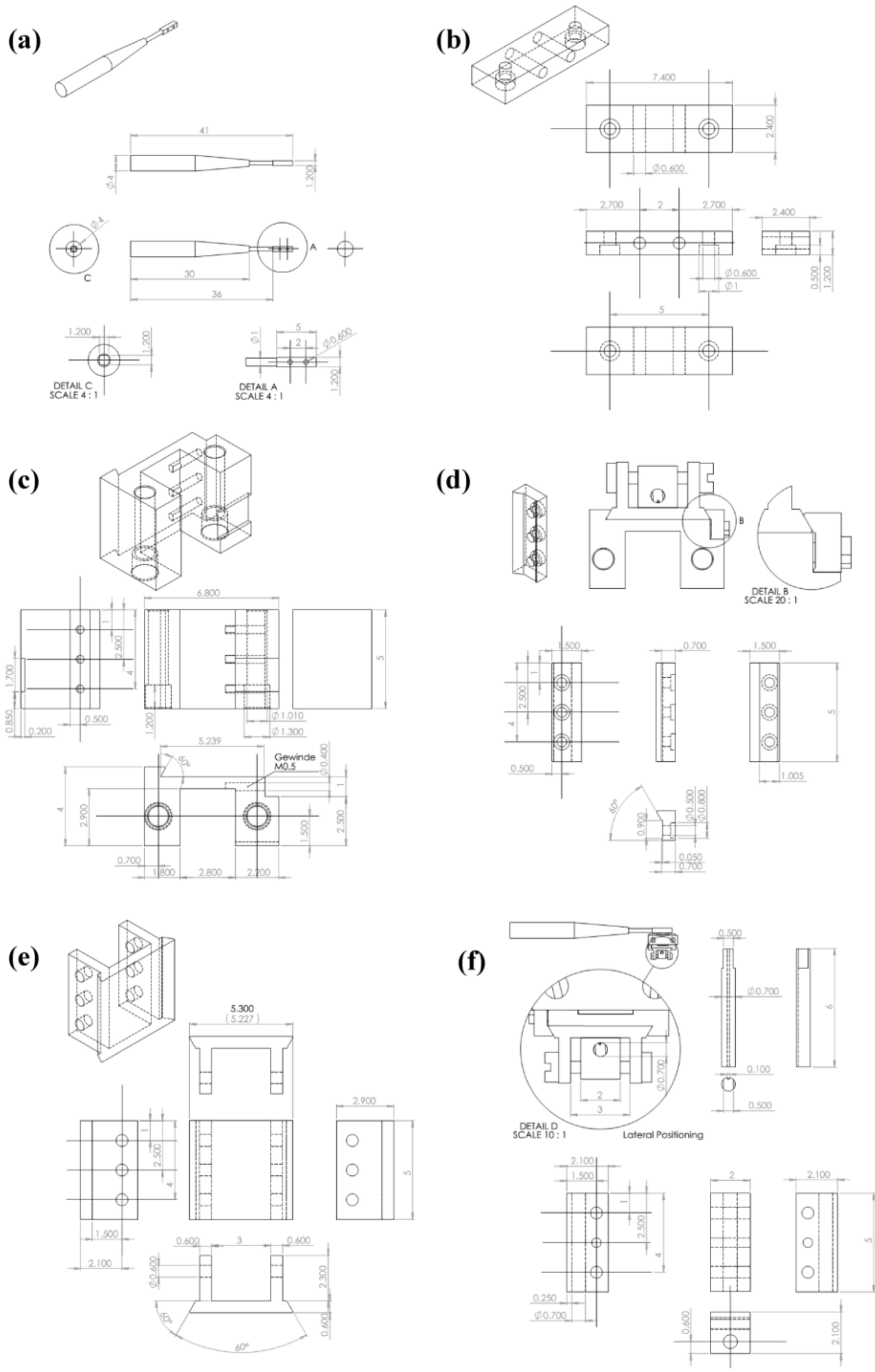

Figure A. Machine drawings (a) microdrive holder (b) mount (c) shuttle (d) shuttle clamp (e) carrier (f) probe holder.

Appendix B. Microdrive assembly

Materials

- microdrive holder 1x
- mount 2x
- shuttle 1x
- shuttle clamp 1x
- carrier 1x
- probe holder 1x
- rod 2x (4.15mm length, 0.5mm diameter)
- spring short 2x (10x1.25x0.10x21 IG)
- berylco tube 1x (0.99mm diameter, 13mm length)
- berylco magnetic tube 1x (0.99mm diameter, 13mm length, manufacturing procedure in next section)
- screws: MO6C 4x, MO5L 6x
- motor (SQL-RV-1.8)
- flexboard
- silver wire (diameter: 0.0127 mm in bare, A-M Systems, https://www.a-msystems.com)
- platinum 10% iridium wire (diameter: 0.0254 mm , California Fine Wire Company, http://www.calfinewire.com)

Prepare magnetic tube: insert 9 ferrite magnets (SMCO YXG-24 Dia 0.73 (-0.03/+0.02) x Length 1 mm (+/-0.02) mm, AIC magnets Ltd., http://www.aicengineering.com) in repelling positions into a berylco tube and solder on top of the tube a berylco plug with M0.6 thread to fix the magnet positions without any gap. In order to provide accurate position readout there should be no missing magnets and no tolerance, nor any gap between the repelling magnets. After soldering the berylco plug, mill the overlapping plug to the same length as the berylco tube without magnets. For the further assembly, two berylco tubes are required, one without magnets and one with magnets, of exactly the same length. When later tightening the M06C precision screw (M06C, Bergeon, http://www.bergeon.ch) to the top and bottom mount it is important to tighten the screw very carefully and not to overheat (e.g. while soldering ground wires) this fragile side of the berylco tube where the berylco plug is soldered in order to avoid damaging or loosening the plug.

Adjust shuttle movements: Connect the two berylco (magnetic and non-magnetic) tubes with precision screws (M06C, Bergeon http://www.bergeon.ch) to the bottom mount. Second, glide berylco springs (CuBe2 FEDER, La Manufacture http://www.lamanufacture.ch) over the tubes (bigger tube holes facing springs) and mount the shuttle. Finally, add the top mount and use precision screws (M06C, Bergeon) to fasten the connection. Move the shuttle up and down: it should move smoothly along the whole range (shuttle movement can be improved by polishing the guide tubes). Make sure the tubes are parallel to each other. Pull the shuttle down, the springs should push it back to the top. The critical step is to verify that the shuttle goes back to the upper most position even for small shuttle displacements near the top. Once satisfactory motion is achieved, remove the top mount and the shuttle.

Prepare shuttle clamp: The clamp has 3 holes that need an additional thread. Use a thread cutter for 0.5 mm screws and cut 2-3 times (turn until the thread cutter penetrates completely through the hole). Test to, attach the shuttle clamp with M05L screws.

Prepare shuttle-motor unit: Hold the motor always at the thread with blunt forceps. Before gluing: clean the shuttle and the back side of the motor with Acetone. The **"**R**"** mark at the motor corresponds to the **"**top**"**, "V" corresponds to the "bottom". When the upper side of the motor (R) is aligned to the shuttle, the lower side will extend a few mm. If the position looks good, take out the motor and apply Torr Seal (Thorlabs Inc, https://www.thorlabs.de) to the bottom and sides of the shuttle (such that none leaks into the motor). Insert and align the motor into the shuttle. Apply pressure on the motor with forceps to guarantee good contact between motor and shuttle, and make sure that the clamp holes are not covered. Let Torr Seal cure overnight. In the next step, add drops of Torr Seal to each corner of the motor-shuttle contact to provide good bonding. Again, let Torr Seal cure for 6 hours and remove excessive epoxy with a scalpel. Attach the flex circuit connection of the motor on top of the shuttle and afterwards on the side (the corner of the prefabricated flex cavity might need to be trimmed). Use fast setting glue (Non-Sag Epoxy, Hardman DOUBLE/BUBBLE 4008, http://www.royaladhesives.com) for these steps. Add very thin layers of solder to the 4 motor pins

Add shuttle clamp: Attach the shuttle clamp to the shuttle (M05L screws): tighten the 2 outer screws so that some force is required to add the clamp, and fix the clamp with the middle screw.

Attach flexboard: Place the board on the shuttle; make sure that there is a tight contact between the board and the motor cable. Add fast setting glue (Non-Sag Epoxy, Hardman DOUBLE/BUBBLE 4008, http://www.royaladhesives.com) to the shuttle frame and the motor block, and then attach the board. Use forceps to apply some pressure to the board. Next, glue the part with the position sensor (NSE-5310) to the shuttle (it is important that it is parallel to the magnet tubes to provide accurate position readout). In the last step, glue the two small pins for clamping the microdrive. Solder the motor connector by applying heat with a soldering iron.

Prepare the carrier: The probe holder is attached to the carrier. First, cut threads for the two middle holes at each side of the carrier and the hole at the probe holder (thread size 0.5 mm). Place the probe holder in the carrier and use two rods (0.5 mm diameter, 4.15 mm length) to fix the holder and enable lateral positioning. Apply drops of glue (Torr Seal) to fix the rods. Attach screws (MO5L, Bergeon) to allow for lateral positioning. Attach the carrier to the shuttle with the clamp.

Top and bottom mount adjustment. Assemble the shuttle-motor unit on the two berylco tubes with the springs (see section "adjust shuttle movements". Small gaps between the motor axis and the top and bottom mounts are necessary to allow for unrestricted movement of the motor. Use sandpaper to remove material at the mounts (total 50 µm gap).

Reference and ground: Solder a reference or ground wire to the screws that attach the bottom mount to the copper tubes, or alternatively, use conductive epoxy (CW 2400, CircuitWorks) to achieve a good connection.

Prepare and attach cable: Braid a copper-omnetics cable (NSD-14 Width 20.0, Omnetics Connector Corporation, http://www.omnetics.com) that is suitable for your needs and that can be attached to a commutator. Solder the cable directly to the headstage board according to the XP1 connector pinout in figure C1 (also figures C3 and C4) and the orientation of the XP1 connector in figure C6.

Motor control: Attach the motor cable to a motor-control box. Use the LabVIEW interface to move the motor along the whole axis. Opening and closing the screws that fasten the top and bottom mounts will adjust the movement. Once smooth movements are achieved, use fast setting glue (Non-Sag Epoxy, Hardman DOUBLE/BUBBLE 4008) to cover the bottom screws so that apart from the tips of the reference wire to avoid contact with the brain or skull. Check every 1000 µm that the measured distance corresponds the real distance travelled.

Appendix C. Circuit Diagrams

The circuit consists of two electrically separated parts. The first part is dedicated to the amplification of electrophysiological signals, it consists of one amplifier AD8605 and three double amplifiers AD8606 with supplementary capacitors and resistors (figure C1). The second part is dedicated to the control of the microdrive, it consists of the piezo motor driver NSD-2101 and the position encoder NSE-5310 with supplementary capacitors (figure C1). Note that the two parts are completely separated and have different grounds labelled AGND for the analog amplification cascade and DGND for the microdrive controller. To decrease noise penetration from the digital part to the analog cascade it is recommended to connect both grounds to the ground of the shielded animal experimental chamber (“global ground”) usually somewhere at the top of the animal cage.

The analog part of the microdrive board is connected to the recording system. In case of metal electrodes this system is a custom-made amplification cascade with its outputs connected to a National Instruments (NI) ADC board, for instance, PCIe-6259. In case of glass pipettes the output signal of the microdrive board is routed to the Axon Instruments AxoClamp-2B via the interface shown in figure C2a, most parts of this circuitry are adopted from the original Axon Instruments headstage schematics. The output of the AxoClamp-2B is routed to a NI ADC card. The digital part of the microdrive board uses the I^2C (Inter-integrated circuit) protocol to communicate with an EX-F320 board (WaveShare Electronics, www. Waveshare.com), via a small interfacing circuit (figure C2b). The EX-F320 board works as a converter from the USB to the I^2C bus. There are many boards with similar functionality on the market. The choice of the current model was dictated by its compatibility with NI LabVIEW and its low price. NI firmware was uploaded to the processor C8051F320 of the EX-F320 board. Interfacing to NI USB-8451 is also possible and can be recommended if there are no financial restrictions. To strengthen the motor we increased the voltage on the EX-F320 board from 3.3 V to 3.6 V by replacing its voltage regulator LD1117-3.3 (ST Microelectronics, www.st.com) with LM1117S-ADJ/NOPB (National Semiconductor, now part of Texas Instruments, www.ti.com) and by using a voltage divider made of a couple of resistors to set the voltage. However, later we have found that 5 V power supply increases the power of the motor even more (powerful motors are beneficial because they can compress stronger springs and therefore provide increased stability of the shuttle). To provide 5 V we disconnected the 3.3 V line from the BUS_B connector of EX-F320 and bridged it with a 5 V bus receiving power from the computer USB port (5 V should be routed to the positive digital power line marked “3V6” in the figures, instead of 3.6 V). Note that the C8051F320 controller of the EX-F320 remains at 3.3 V, it has open collector outputs to drive I2C which work well with a 5 V bus voltage. The original NI USB-8451 can be used with 5 V powering directly without modifications.

To remove possible ground loops and decrease noise, we attached EX-F320 to the computer through the USB optical insulator USB-ISO (Olimex Ltd, www.olimex.com). Both EX-F320 and USB-8451 do not have 2.7 K pull-up resistors and 100 pF noise suppressive capacitors in their SDA and SCL lines of the I2C bus. These parts should be added manually (figure C2b). Additionally, a pair of overvoltage/under-voltage protective diodes for each line can be useful to protect the microcontroller port from occasional voltage peaks that can be harmful for the chip.

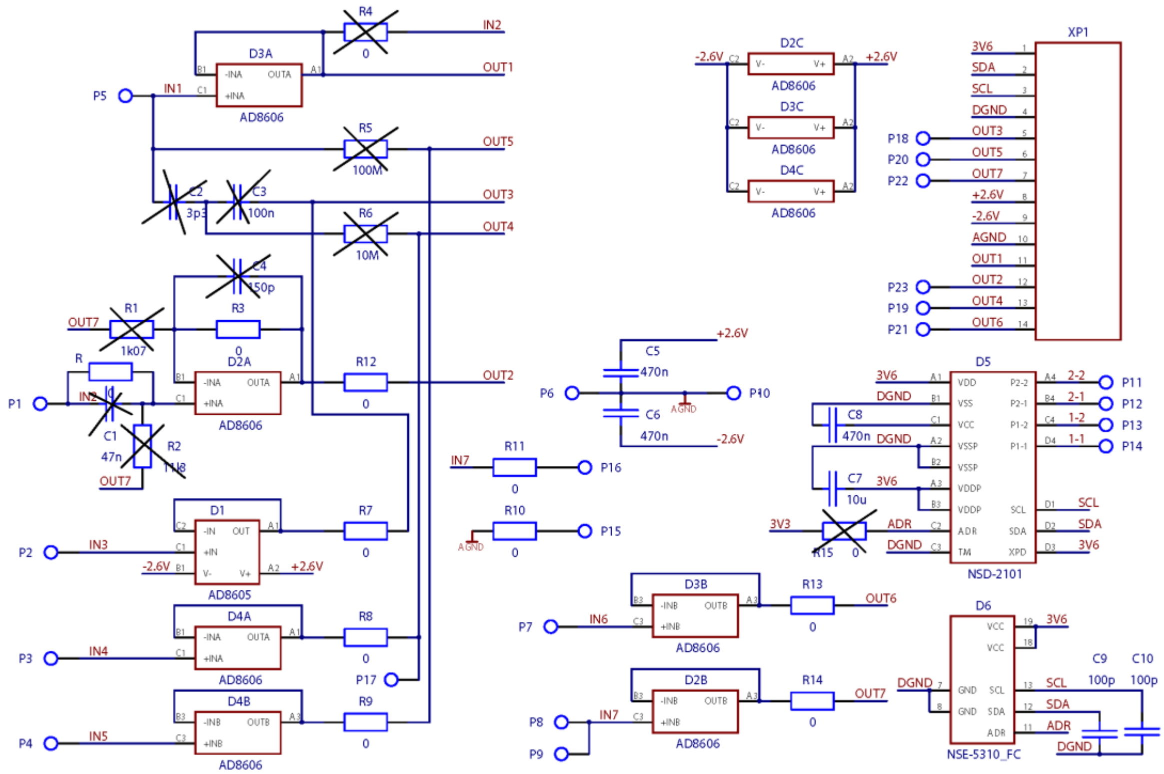

Figure C1. Electrical circuit diagram of the microdrive board containing seven unity gain followers (AD8605/AD8606) for boosting electrophysiological signals. Non-soldered elements and circuit parts are crossed. Electrode and reference signals are connected to pads P1-P5 and P7, P8.

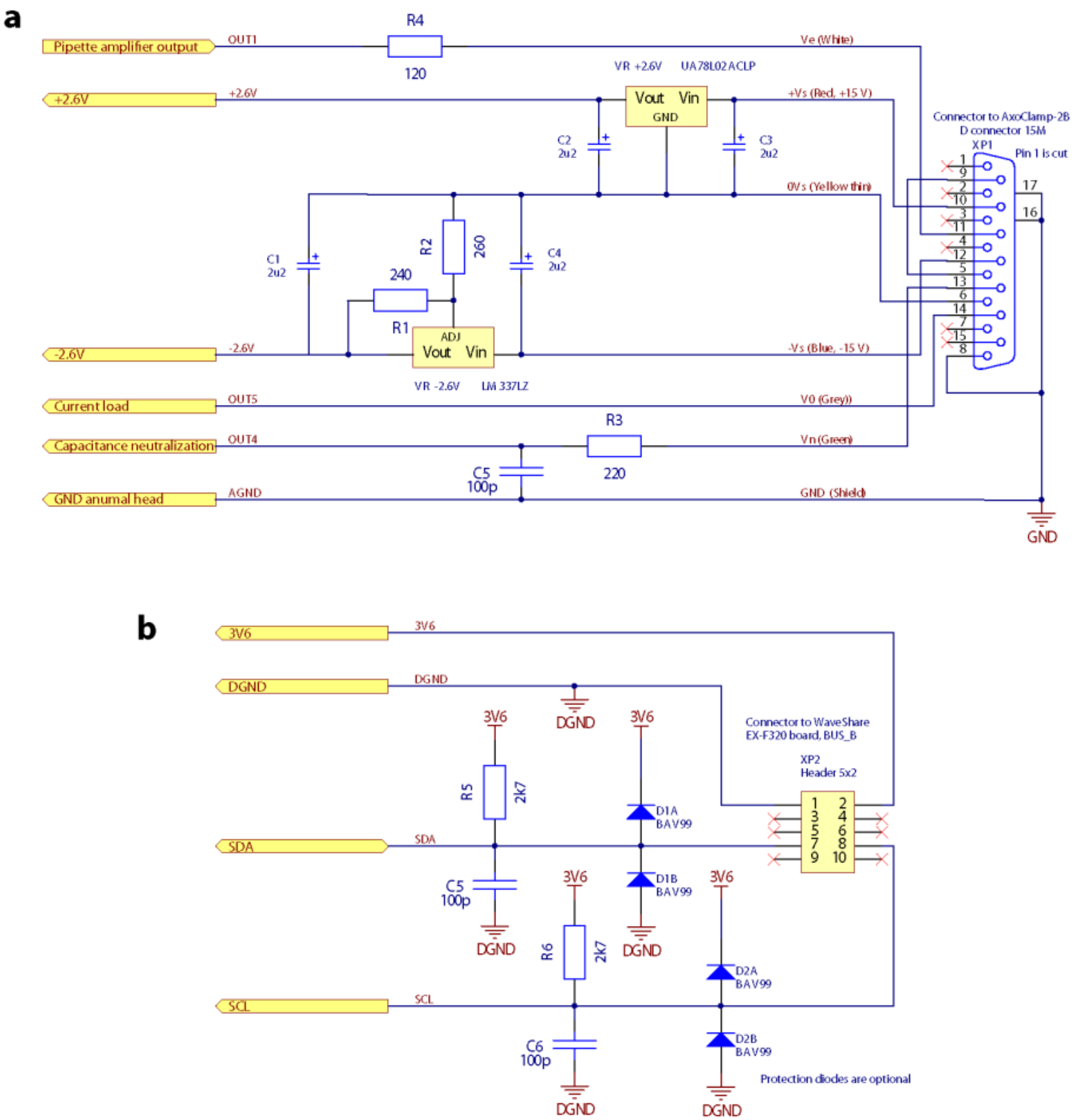

Figure C2. Electrical circuit diagrams of interface boards needed for connecting the microdrive board with (a) the AxoClamp-2B and (b) the EX-F320 USB-to-I2C interface board.

The seven amplifiers AD8605/AD8606 can be operated in three different configurations (by exchanging passive components and using zero-ohm resistors).

In the first configuration shown in figure C1 all amplifiers are configured as unit-gain followers. They can be connected independently to seven high-impedance signal sources (electrodes, etc.). The output impedance of these amplification cascades is low. Thus, the signals will be less affected by environmental noise during transmission from the animal head to the recording equipment even though their amplitudes remain unchanged. This configuration can be used with numerous electrodes for recording electrophysiological signals of different nature, such as EEG, EMG, ECG, as well as intracranial LFPs and neuronal activity. The unit-gain followers can also be used for boosting signals from other sensors, such as thermistors for measuring temperature of the brain, pressure sensors for measuring respiration, and many more. The second configuration is created for recoding from one metal electrode with improved signal conditioning. In this configuration shown in figure C3 the signal is band-pass filtered (0.3-10 kHz) and amplified 101 times. The third configuration shown in figure C4 is developed for intracellular recordings with high-ohmic glass pipettes.

Neural signals recorded with metal electrodes have typical amplitudes in the range 100-300 μV. To decrease the influence of external noise in transmission lines it is desirable to amplify the neural signals as early as possible, preferably on the animal’s head. However, one should not amplify the DC offset of the signal arising from the galvanic potential between the electrode and the brain. This offset can be in range of 0.4 V and its amplification can lead to signal clipping to rails of the amplification cascade. Thus, the signal should be high-pass filtered before amplification. Stability of operational amplifiers can be affected by high-frequency noise. Also, the signals will be digitized properly only when their spectrum lies below the Nyquist frequency (1/2 of ADC sampling rate). For these reasons the bandwidth of amplification cascade is usually limited artificially. To satisfy these requirements we realized 0.3-10 kHz band-pass filter on the headstage with amplification coefficient 101 for one electrode (figure C3). The signal from that metal electrode is first routed to the unit-gain follower D3A. Then it goes through a passive high-pass filter constructed from a 47nF capacitor C2 and a 11.3 kΩ resistor R2 providing -3 dB attenuation at 300 Hz. To filter the signal relatively to the reference electrode, the output of the unit-gain follower from the reference electrode D2B is connected to the lower end of the resistor R2. Then the signal goes to the non-inverting input of the operational amplifier D2A that amplifies the difference between high-pass filtered output and reference electrodewith gain 101 (because of the voltage divider consisting of 1.07 kΩ resistor R1 and 107 kΩ resistor R3). Note that this is not a true differential cascade and the attenuation of common-mode disturbances will be only about 100x (-40 dB). The 150 pF capacitor C4 in the feedback limits high frequencies to around 10 kHz (-3 dB). The remaining four amplifiers D1, D3B, D4A, and D4B can be used as unit-gain followers for other signals as described earlier. A small galvanic DC offset of the reference electrode is present in the output which can be subtracted during data analysis.

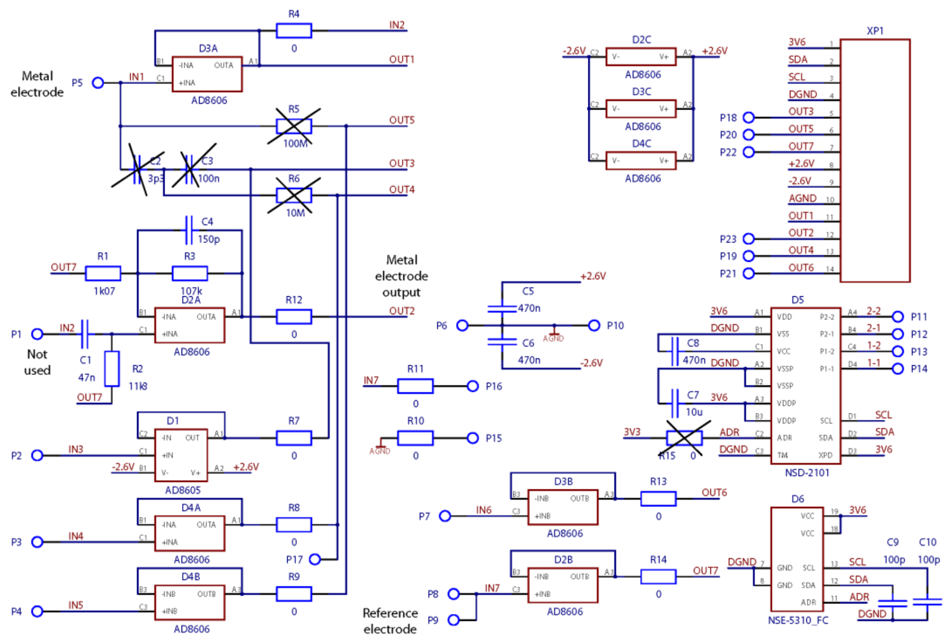

Figure C3. Electrical circuit diagram of the microdrive board containing amplifiers for metal electrode and reference electrode, as well as four high impedance unit-gain followers for signal boosting.

One core feature of our headstage is the possibility of recording intracellular potentials using a glass pipette and an AxoClamp-2B patch-clamp amplifier (Axon Instruments). The schematics of this headstage variant are shown in figure C4. The base of the intracellular amplifier is the unit-gain follower D3A. The electrode-cell junction can be loaded with a current provided through resistor R5. For that resistor we chose a value of 100 MΩ, which is well suited for 30-300 MΩ pipettes such as the one used to produce figure 7. If the impedance of pipette is lower than this range, a 10 MΩ resistor should be used instead (a 10 MΩ resistor has been used for the juxtacellular recording with a 14 MΩ pipette shown in figure 6).

The capacitance of the input cascade in the range 1-3 pF can affect the shape of intracellularly recorded spike waveforms. To diminish such influence, an input-capacitance neutralization circuitry has been implemented in the AxoClamp-2B. The output of this circuitry is connected through a 3.3 pF capacitor C2 to the silver wire leading into the pipette. However, unwanted current leakage is possible through the dielectric of this capacitor. To decrease possible leakage, the opposite plate of capacitor C2 is connected by a 10 MΩ resistor R6 to the output of the unit-gain follower D3A (connection OUT1-OUT4 in connector XP1). The constant potential of the capacitance neutralization circuitry of the AxoClamp-2B is isolated by a 100 nF capacitor C3. Optional low-leakage diodes CMPD60015 connecting amplifier inputs with the outputs are introduced for overvoltage protection of the input as needed during pipette clearing (“buzzing” used to disrupt cell membranes), when the potential of the electrode in the pipette can jump up to ±160 V relatively to ground. The amplifier power supply lines should follow this potential because the amplifier used in the original Axon Instruments headstage has maximal power supply range of ±15V. There is special circuitry inside the AxoClamp-2B that lets the power supply voltage follow the electrode potential. This circuitry is based on a couple of pull-push high-voltage transistors and circuitry for maintaining a difference between transistor outputs below 30 V. Unfortunately, amplifiers with ±15 V power supply voltage were not available in small form factor. Thus, we used the compact AD8606 amplifier with power supply of ±2.6 V. To adopt it to the ±10 V power supply of the AxoClamp-2B, we had to introduce two additional voltage regulators UA78L02ACLP and LM337LZ shown in figure C2a. These voltage regulators keep the power supply lines within ±2.6 V relatively to the AxoClamp's 0 V output that jumps up and down as needed. Unfortunately, we found that the AD8606 amplifier does not work well with heavy capacitive loads. To decrease effect of loading this output by the cable capacitance, a 120 Ω resistor R4 has been introduced. The value of this resistor may need adaptation depending on the length of the cable and its capacitance. The original Axon Instruments headstage also had a similar resistor, but its value was 22 Ω. If the value of this resistor is too small, parasitic oscillations can be observed. If it is too high, the shape of the spike can be affected by the resulting low-pass filtering. The low-pass filter in the capacitance neutralization line, constructed from a 220 Ω resistor R3 and a 100 pF capacitor C5, is used to suppress high-frequency noise.

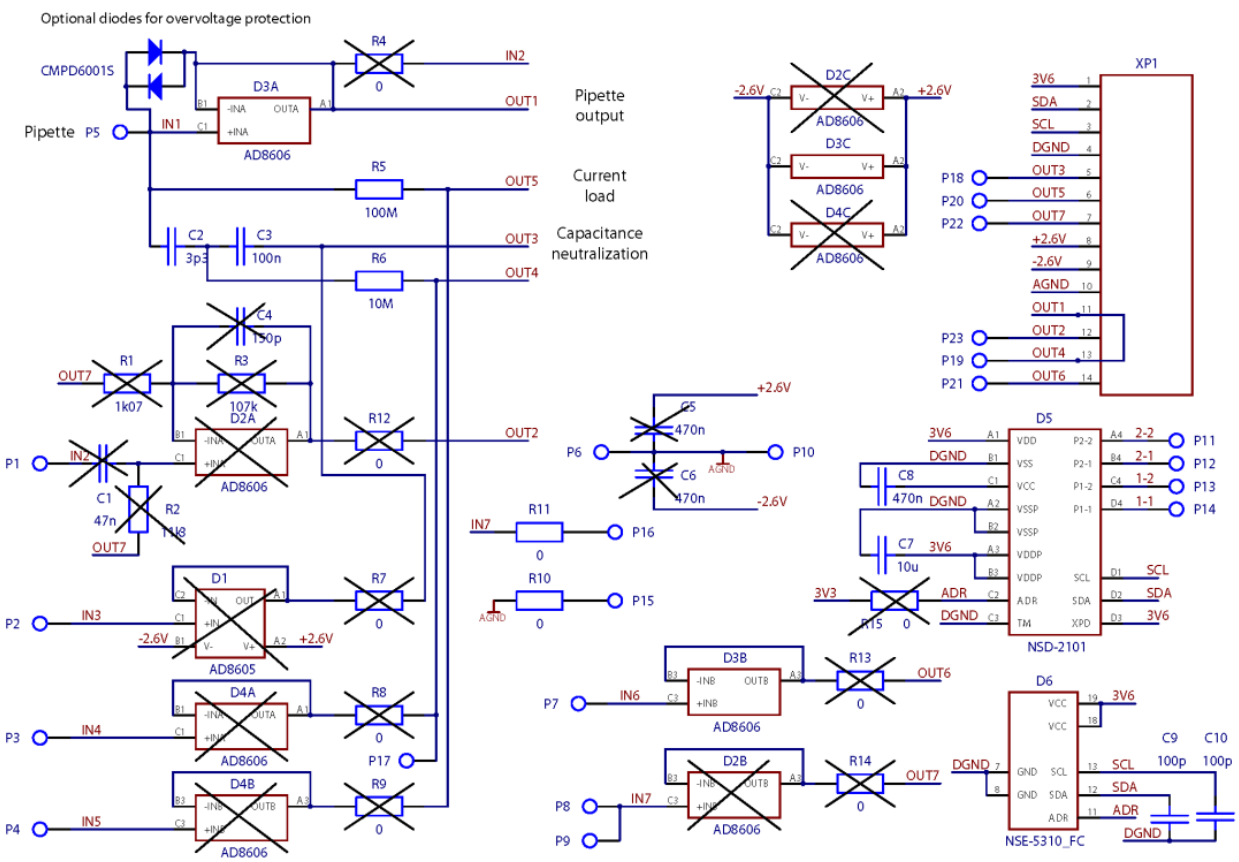

Figure C4. Electrical circuit diagram of the microdrive board configured for glass pipette electrodes and circuitry connected with the AxoClamp-2B patch-clamp amplifier. Note that the black-crossed amplifiers cannot be used in this configuration because they are powered from the same power lines ±2.6 V as the pipette amplifier. These lines jump up and down during pipette cleaning ("buzzing") up to ±160 V.

Our headstage consumes more current that the standard Axon Instruments headstage. This is caused mainly by the several amplifiers powered by AxoClamp-2B and by the additional voltage regulators. For this reason we increased the original AxoClamp-2B current limit from about 7 mA to 14 mA by putting four 82 Ω resistors in parallel with existing current-controlling resistors (figure C5). There are four resistors because there are two independent cascades (ME1 and ME2) and because power supplies are bipolar (±10V). In the cascade ME2 two large current-controlling transistors are placed on one common heat sink. When current doubles relative to normal levels, the cooling from this heat sink may be insufficient. As a workaround, one transistor can be removed from the heat sink and placed on its own heat sink of similar size. We elevated the old heat sink above the board to provide space for the new one.

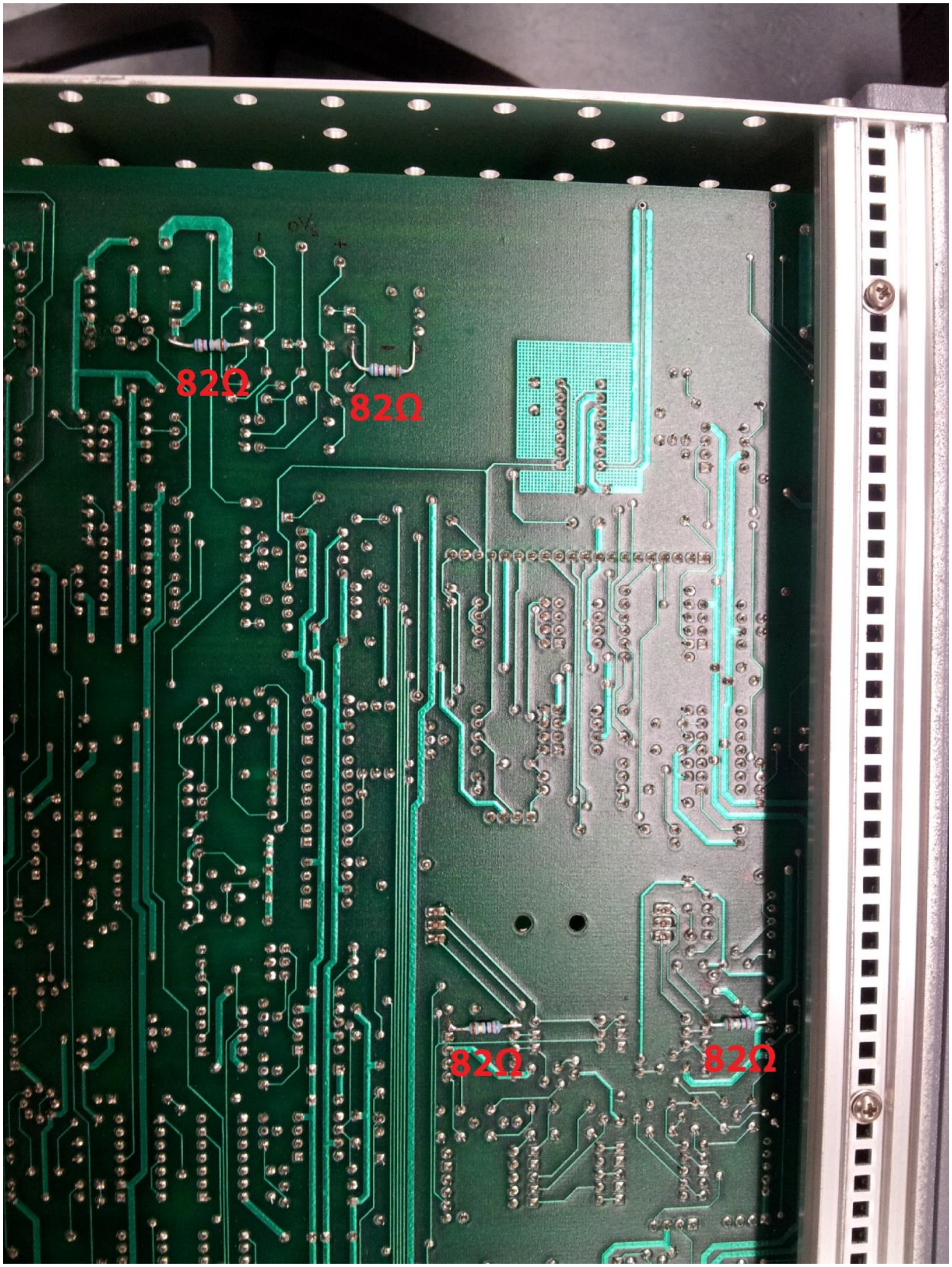

Figure C5. Photo of the AxoClamp-2B main board with four additional 82 Ω resistors increasing power supply current limit up to 14 mA needed for our headstage.

The following three figures show placement of components on the microdrive board (figure C6), routed signals in four layers of this board (figure C7), and the photo of the position sensor NSE-5310 in bare die form attached to the microdrive board by classical wire bonding (figure C8). In the final version of the board we used flip chip technology with direct connection of die pads to the board, this technology eliminated the space-consuming wire bonds shown in figure C7 and allowed us to place the sensor on the side of the microdrive shuttle.

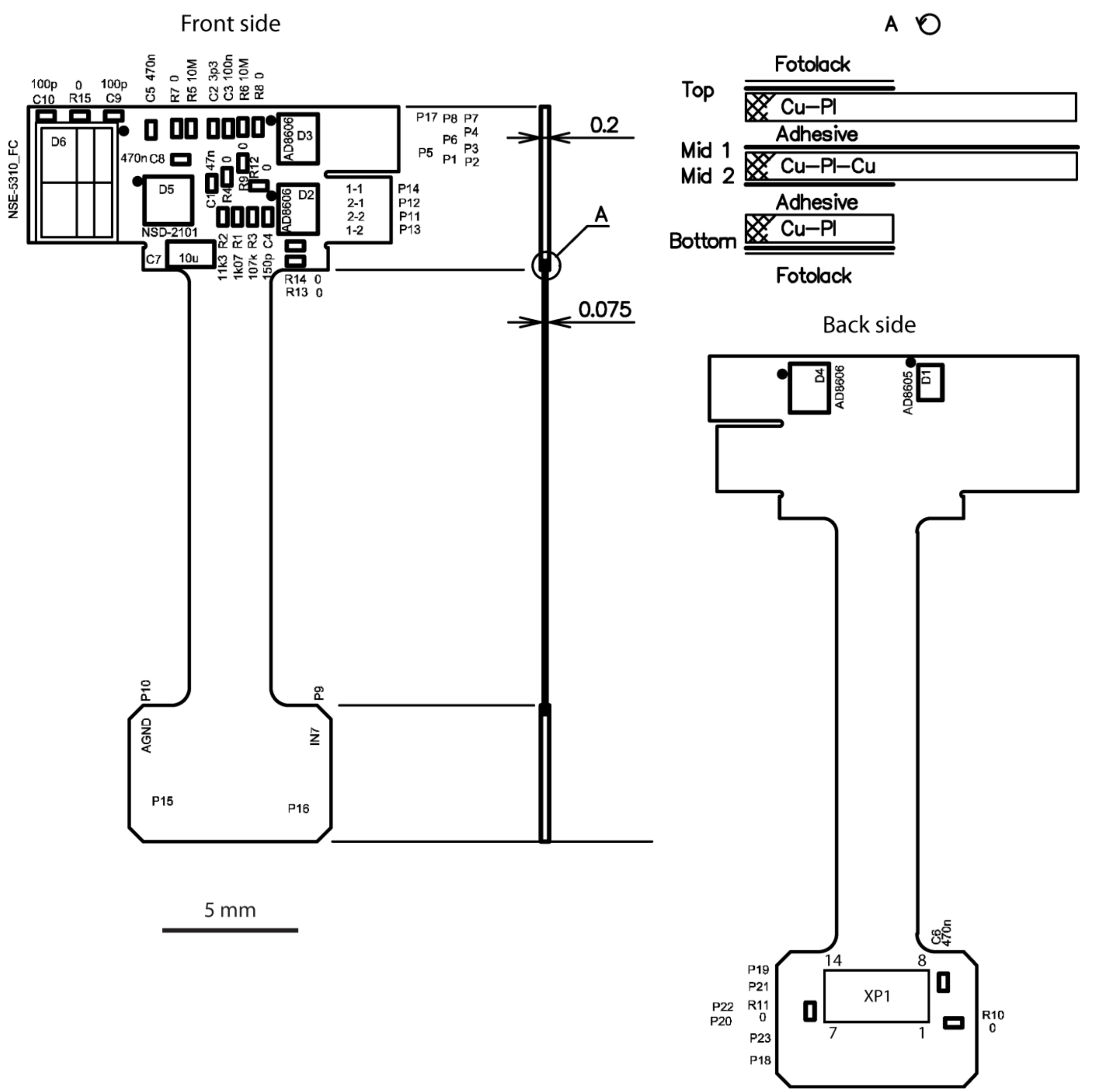

Figure C6. Placement of components on the microdrive board. All passive components have form factor 0201 except the 10 μF capacitor C7 that has form factor 0603.

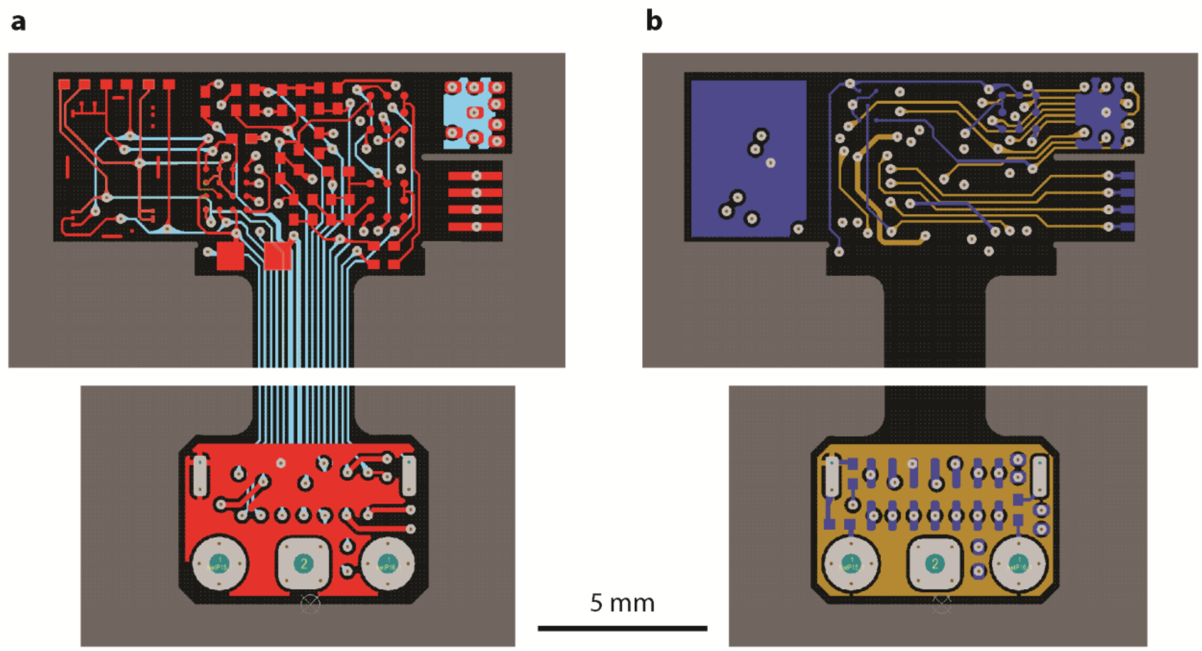

Figure C7. The signal wiring on the microdrive board is done in four layers. To show the board endings at higher magnification, the flexible single-layer part of the board has been truncated for display purposes. (a) Top and middle-1 layers. (b) bottom and middle-2 layers. The top layer is shown in red, middle-1 layer in light blue, middle-2 layer in yellow, and the bottom layer in violet.

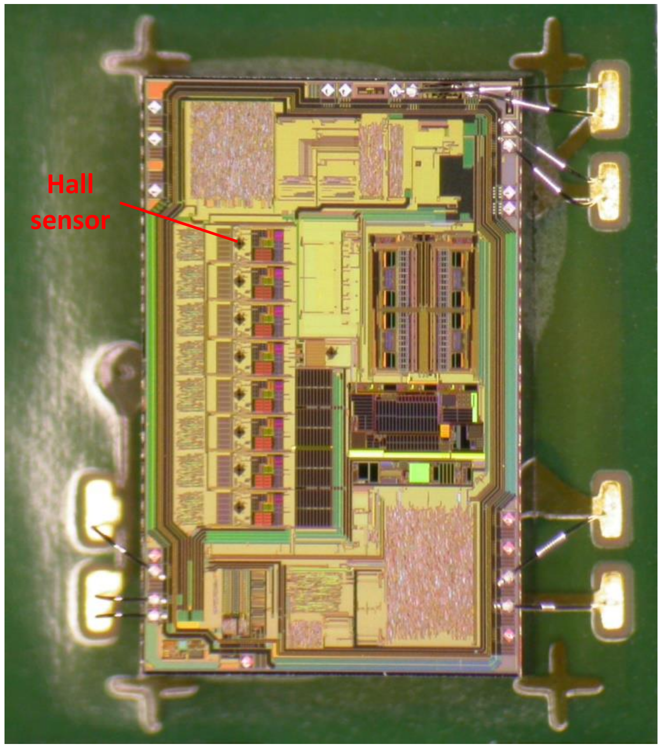

Figure C8. The position sensor NSE-5310 in bare die form attached to an early version of the microdrive board by classical wire bonding. The eight identical vertically aligned dark square areas on the left side of the die are the hall sensors measuring the strength of the magnetic field along the stack of magnets in the berylco tube. Because wire connections of die pads with PCB pads would have required more space than available on the side of the microdrive, we made use of space-saving flip chip attachment of the die to the PCB. The die size is 3.98 × 2.57 mm.

Appendix D. Microdrive control software and graphical user interface (GUI)

We wrote a custom LabVIEW program for controlling the microdrive using a closed feedback loop. The main features of the software and the functionality of the graphical user interface (GUI) are presented in the following.

When the motor control software is launched, the user is asked to set some parameters, such as the folder in which to save the log file (logged are the allowed movement range, the target positions, the speed settings, the current position, and the zero position). A set of debugging controls can be selected for running the software without communication with either the motor and/or the sensor, which is a feature that can be helpful when trying to isolate hardware malfunctions. After setting these initialization parameters, the runtime display appears. The runtime display is split into two parts (figure D): on the left, important status information can be inspected and on the right are controls and indicators that are less frequently used (the runtime display can be conveniently dragged beyond the right edge of the screen to save screen area).

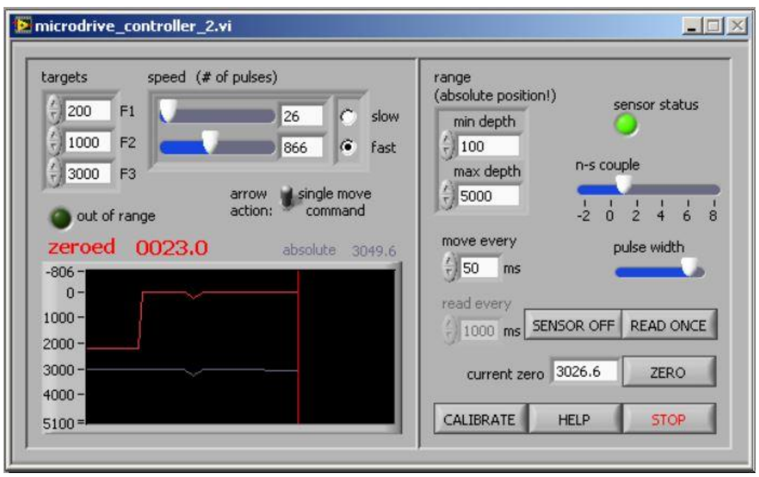

Figure D. Graphical user interface of the motor-control software. On the left, movement speed and targets can be manually set. The red line indicates the electrode position as a function of time, reported in μm relative to the brain surface. On the right, less frequently used features can be accessed, such as the n-s couple slider and the sensor status (green).

Using the controls on the right, it is possible to set the electrode range beyond which no movement is allowed. The sensor status indicator (green-red indicator, right) reports whether the sensor readings are reliable, typically the indicator turns red (negative status value) only in case of serious malfunction or an incorrectly assembled microdrive. The n-s couple indicator (right) indicates the magnet number where the sensor is currently located. That number can be manually changed when using a new microdrive. Note that after stopping and restarting the control software, the current magnet number value is calculated from the last position that was stored in the log file. Whenever the value of the pulse width slider (right) changes, the corresponding write command is sent to the motor.

Two numeric controls (right) allow choosing the period at which to write move commands to the motor and the period at which to read values from the sensor. Because every such event may produce noise in the electrode signal, the sensor should be mostly off during experiments. By default, after every move command, a sensor reading is triggered automatically.

The 'zero' controls (right) allow subtracting a fixed number from the absolute electrode position. Typically, this feature is used to subtract the relative position of the surface of the brain to obtain electrode depth information. Pressing the button sets the current position to zero and displays the position (in previous coordinates) next to it. The zero position can also be manually set in the text field. The most recent zero position is saved in the log file and loaded at each launch.

On the left side of the runtime display the user can set two different speeds defined by the number of pulses delivered after each move command. Pressing the keyboard arrows up/down triggers electrode movements with the 'slow' speed setting, whereas right/left key presses trigger movement with the 'fast' speed setting. When the arrow action is set to continuous movement, holding down the arrow keys will continuously send move commands until the button is released or the electrode is out of range.

Three target positions (zeroed) can be defined and reached by holding the corresponding keys F1-F3 on the keyboard. Pressing the F1-F3 keys sends continuous move commands towards the target with the pulses settings specified in the speed control. Releasing the F1-F3 keys stops the movement.
NOTE: when holding one of the target keys, the motor receives move commands until the current position crosses the target. At low speeds (i.e., a few micrometers per move command), the electrode stops very close to the target. Note that however, if a move command generates a large movement, then the electrode might overshoot the target by tens of micrometers.

The microdrive control software is implemented as a LabVIEW state machine with 6 states (init, wait, check, read log, run, exit). In the run state, normal execution is handled by two parallel while loops, one for the Hall sensor and the other for controlling the motor. The sensor loop is simple: if a reading is requested (explicitly by the user, or after a change of parameters, or a move command), 5 bytes are read and decoded from the sensor and the runtime display is updated. The motor loop includes an event structure which listens for user input:

1. Whenever there is no user input within a short time window (default 50 ms), the timeout case reads the last measured electrode position (zeroed) and stores it in a functional global. A check is performed to verify that the current position is within the allowed range.
2. If an arrow-key press is detected, the corresponding speed (fast for left-right, slow for up-down keys) and direction are set. Then, if the electrode is within the allowed range, one or several move commands are issued, each followed by a reading from the sensor.
3. If the 'pulse width' parameter is changed, the new value is imply written to the motor.
4. If the value of one of the two 'speed' sliders is changed, the newly selected speed will be used for the next move command.
5. If the user presses the 'read once' button, a user event is generated that requests one reading from the sensor loop.
6. If continuous reading from the sensor is switched on or off, the 'read every' control is enabled/disabled accordingly.

7. If the 'calibrate' button is pressed, the user is first warned that some movements will be generated and, if accepted, three calibration steps will be performed (see datasheet). During calibration, continuous reading from the sensor is automatically switched on.
8. If the user changes the 'n-s couple' value and confirms the change, the index of the current magnet is updated. A single reading from the sensor is then triggered to update the position value.
9. If the user presses the 'zero' button and confirms the choice, the current position is set to zero, the new zero (in old coordinates) is saved in the log file, and a single reading is triggered to update the position.
10. If the user changes the value of the current zero, that value is saved in the log file and a single position reading is triggered.
11. A change of the allowed range will update the range in the position graph accordingly.
12. The help button opens a simple guide window.
13. The stop button closes the motor loop, sends a close signal to the sensor loop, and activates the exit state in which all the necessary information is saved to the log file.