Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information for Spatiotemporal norepinephrine mapping using a high-density CMOS microelectrode array

Pt electrode structure and fabrication

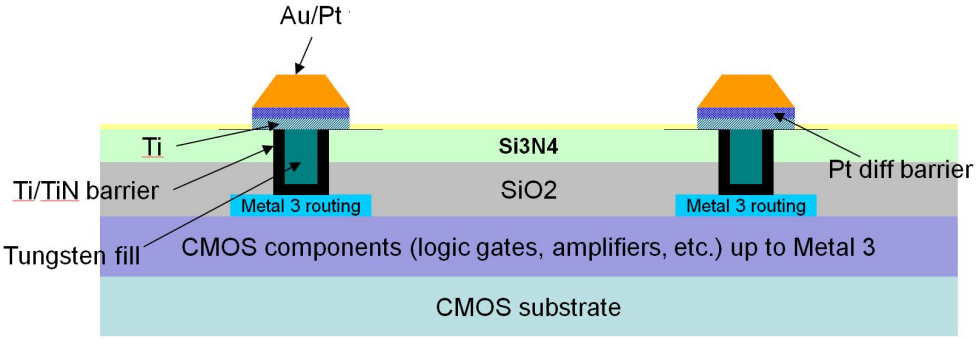
The process of forming the Pt electrode in the electrode array chip is as follows:

1. The process starts with a lightly doped p-type silicon substrate. (The CMOS process used for the chip is an n-well CMOS process);
2. All on-chip electronic components (logic gates, amplifiers, etc.), including metal routing patterns to connect electrodes on Metal 4 to the I/O pads, are manufactured up to Metal 3 using the standard CMOS manufacturing process;
3. Chemical and mechanical polishing (CMP) is performed to make the post-Metal-3 surface planar;
4. SiO_2 and Si_3N_4 layers are deposited on the surface of the chip. Si_3N_4 acts as the passivation layer;
5. Chemical and mechanical polishing (CMP) is performed to make the surface planar;
6. Etching is performed to allow the formation of contacts from the passivation layer surface to the Metal 3 routing pattern at the base of the electrodes formed in step 2;
7. A Ti/TiN barrier layer is deposited inside the contact well to provide a buffering layer for tungsten;
8. Tungsten is deposited to fill the contact well to form the vertical contact column to the raised electrode on the passivation layer surface;
9. The base of the electrodes at the passivation layer surface using a thin platinum diffusion barrier on the order of tens of nm. This diffusion layer makes electrical

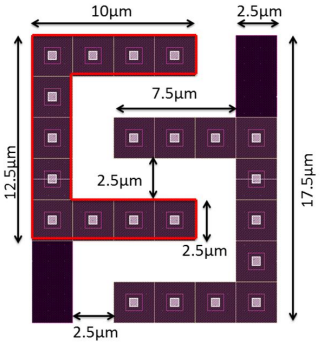
contact to the tungsten plug as well as acting as the base layer for Au/Pt tip of the electrodes;

10. The body of the electrode tips on the chip surface is formed using Au with Pt coating surrounding the tips. The height of the tips from the chip surface is 1.5 µm. The electrodes were intentionally designed to rise above the surface to allow better contact between the electrode and tissue slices that will ultimately be studied.

Supplementary Figure 1 shows the cross section of the electrodes manufactured using the steps described above (not to scale). Supplementary Figure 2 shows the top view of one pair of working electrodes with dimensions clearly marked.



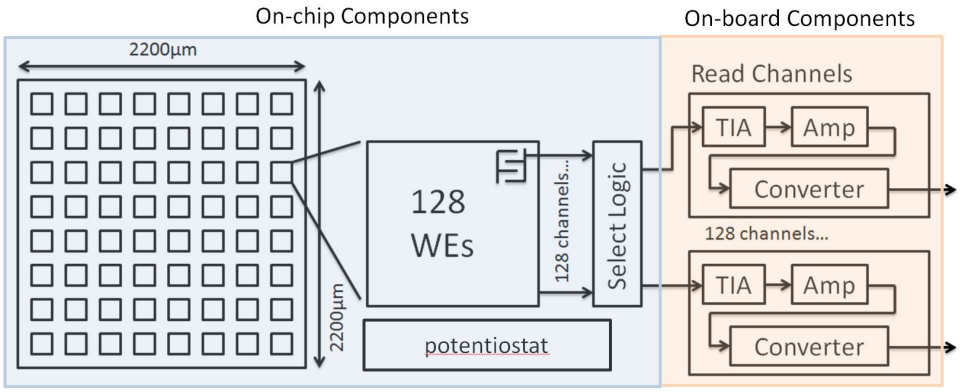
Supplementary Figure 1. Cross section of the Pt electrodes (not to scale).



Supplementary Figure 2. A pair of working electrodes designed with an “F” shape.

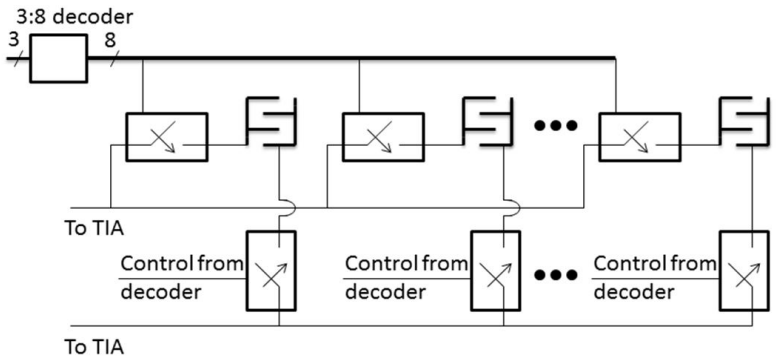
On-chip circuits

The electrode array was fabricated with on-board logic and a potentiostat circuit capable of addressing 128 electrodes simultaneously. The on-chip circuits consist of an 8,192-electrode array, electrode subarray selection logic, and a potentiostat circuit for the on-chip RE, CE, and WEs as shown in Supplementary Figure 3.



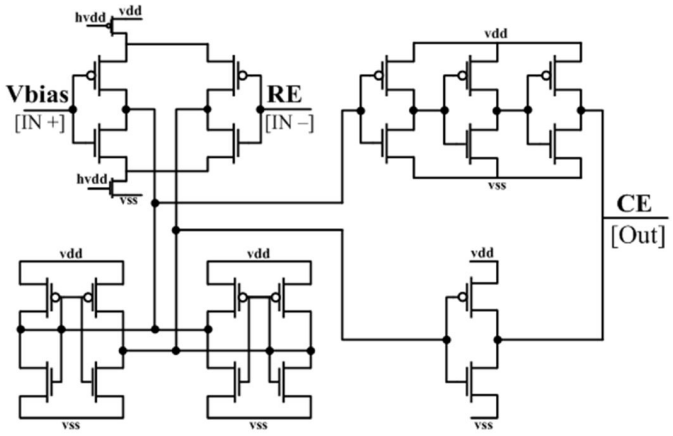
Supplementary Figure 3. On-chip components and how they connect through select logic to 128 on-board read-channels.

Each electrode in each subarray is connected to a switch that will be turned on or off by a 3:8 decoder. The switches are then connected out to one of the 128 transimpedance amplifiers (TIAs) in the on-board read-channels, as outlined in Supplementary Figure 4. Each of the electrode pairs in the figure represents one 128-electrode subarray, of which there are 64 total. Although there are separate switches for each electrode in a pair, both the vertical and horizontal switches are controlled by the same decoder so that all 128 electrodes of a single subarray will be on at the same time.



Supplementary Figure 4. Control logic for the electrode subarrays.

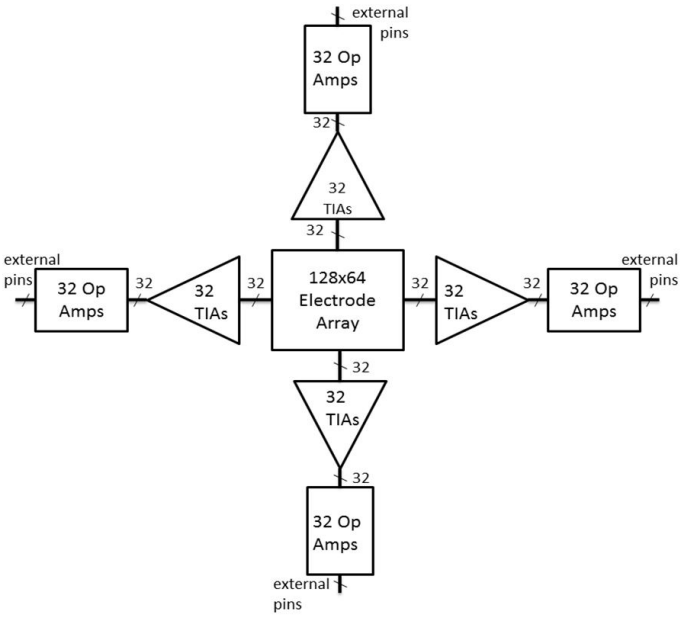
Although each subarray has its own reference and auxiliary electrode, only one potentiostat was needed for the entire design. The main goal of the potentiostat is to maintain a constant potential between the working electrodes and a RE while supplying current to a CE. A bias voltage is applied to the RE and the corresponding induced current will describe the concentration of the chemical present at the working electrode. Each of the auxiliary and reference electrodes found in the subarrays is connected together so that only one potentiostat is required to control the entire array. The potentiostat is designed to operate up to 10,000 V s^{-1} for cyclic voltammetry and in the range of -1 V to +1 V for amperometry. The single op-amp structure used for the potentiostat is based on our proposed self-biased inverter based design[41] and is shown in Supplementary Figure 5.



Supplementary Figure 5. A self-biased inverter based potentiostat circuit.

Board-level circuits and system setup

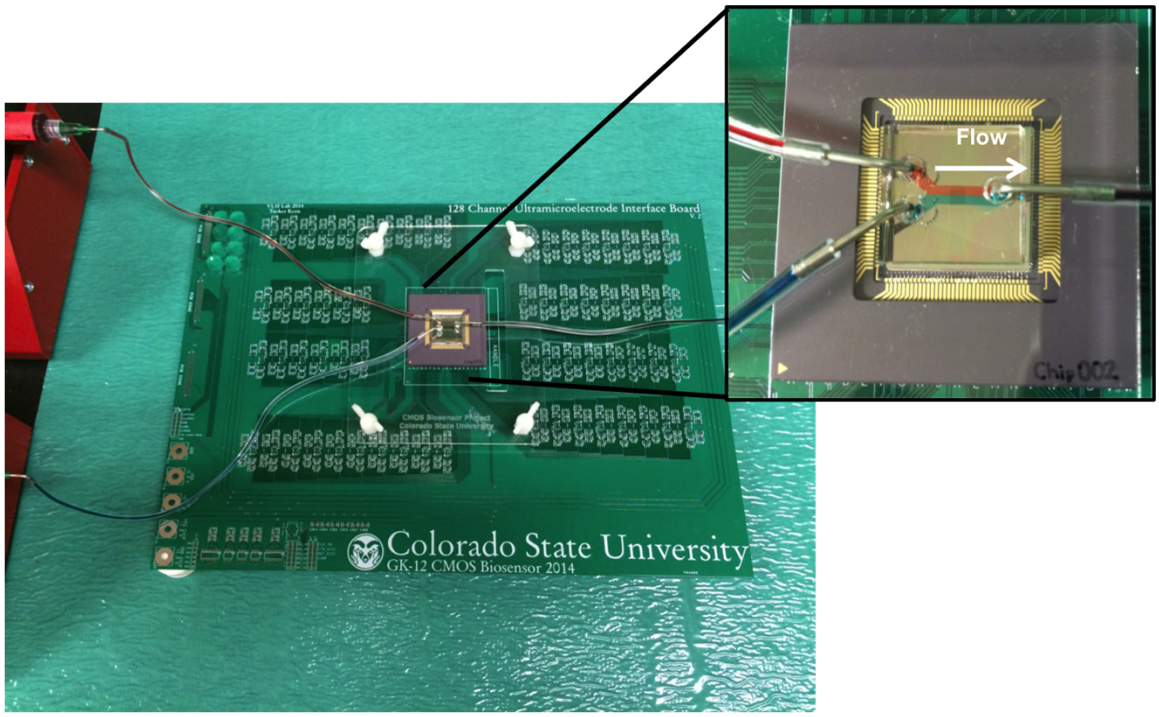
The outputs from 128 selected electrodes are connected to 128 read-channels on a circuit board. Each read-channel on the circuit board consists of a transimpedance amplifier (TIA) and a main amplifier. The transimpedance amplifier has a gain of 10 M□. The purpose of the main amplifier is interfacing with the analog-digital converter used. The main amplifier has a gain of 2. Supplementary Figure 6 shows the organization of the board-level components. The block in the middle is the custom electrode array chip. The read-channels receive the current and voltage signals from the 280-pin package on the board for amperometric and voltammetric operations, respectively.



Supplementary Figure 6. Main board-level components.

The circuit board is manufactured by Advanced Circuits Inc. with 4 layers of signals routing. In addition to 128 read-channels, the circuit board also contains voltage regulators, control logic for the custom electrode array chip and for the data acquisition board on the host computer. Supplementary Figure 7 shows the circuit board for the 8,192-electrode array system. The 128-channel output from the circuit board is sampled by a data acquisition board through a host PC. The data acquisition board that works with the electrode array circuit board is an ADLink 2200 series 64-/96-CH high performance multi-function data acquisition card. Data acquisition from 128 data sources is triggered by the electrode array circuit board. A suite of Matlab functions have been developed to provide interface between the electrode array chip, the on-board read-channels, and the data acquisition board inside the host PC. Custom signal processing functions were also developed in MATLAB and C++ for generating electrochemical images from the raw data acquired by the system.

Supplementary Figure 7. Circuit board with the electrode array chip in the middle and other supporting circuits around it.



Supplementary Figure 8. Setup for microfluidic experiments. The syringe pumps at the left pump two different fluids into the Y-shaped microfluidic channel positioned across the chip (inset image). The chip is connected to the PCB, and the microfluidics are compressed onto the chip using a custom-cut PMMA plate with nylon screws.

Supplementary Table 1: CMOS chip specifications.

Specification	**Value**
Electrode Pitch	30 μm
Subarray Dimensions	250 μm × 250 μm (128 electrodes)
Array Dimensions	2 mm × 2 mm (8192 electrodes)
Maximum Experimental Data Collection Rate (Single Subarray)	100 Hz
Maximum Experimental Data Collection Rate (Entire Array)	0.0156 Hz

Supplementary Video 1: Electrochemical heat map video of norepinephrine diffusion across a subarray. The 10-μL drop of 100 mM norepinephrine was added at the top left corner of the subarray and diffused to reach a final concentration of 4.8 mM. An oxidation potential of +0.6 V (vs. Pt) was applied, and current was monitored at each electrode at a rate of 100 Hz. In the heat map, red indicates high current (12 nA) and blue indicates baseline current (0 nA).

Supplementary Video 2: Optical video and corresponding electrochemical heat map video of norepinephrine transport across the array. The microfluidic channel with dimensions 82 μm × 2.5 mm × 6.0 mm was positioned across the array with fluid contained in the 5-mm diameter reservoirs at each end. The blue fluid in the left reservoir is neurobasal media and the red fluid in the right reservoir is neurobasal media containing 10 mM norepinephrine. After additional solution is added to the right reservoir, differences in head pressure result in norepinephrine transport across the array. An oxidation potential of +0.6 V (vs. Pt) was applied, and current was monitored at each electrode in a subarray at a rate of 100 Hz, cycling between subarrays at a rate of 1 Hz. In the heat map, red indicates high current (16 nA) and blue indicates baseline current (0 nA).

Supplementary Video 3: Optical video and corresponding electrochemical heat map video of bimodal distribution generated using a microfluidic system. Fluid transport is achieved using syringe pumps with a total flow rate of 100 μL min^{-1}. The blue solution is neurobasal media and the red fluid is neurobasal media containing 10 mM norepinephrine. The position of the fluid interface was controlled by varying the flow rates of the two inlet pumps. An oxidation potential of +0.6 V (vs. Pt) was applied, and current was monitored at each electrode in a subarray at a rate of 100 Hz, cycling between subarrays at a rate of 1 Hz. In the heat map, red indicates high current (200 nA) and blue indicates baseline current (0 nA).