CMOS Low Current Measurement System for Nanopore Sensing Applications

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***Abstract*—We present a 16 channel micro-chip implementation of a low current measurement system for use in automated nanopore sensing applications using capacitive feedback. We successfully used our device to perform voltage-clamping and current measurement across specially designed nanopores for low voltage sensing to detect the presence of nanoparticles. Nanoparticle events in the pore can be seen as transient changes in current from the standing current. We demonstrate our system by detecting events from 2 ms to 50 ms with a 250 pA to 350 pA change from the baseline current.**

I. Introduction

Synthetic nanopores, which have diameters of a few nanometers, are gathering large interest for their potential applications in biological sensing including the detection of nanoparticles through electrical current-voltage (I-V) measurements. When a passing molecule interacts with the inside of a pore, it can be detected by a transient change in current with a voltage clamping measurement. The duration and amplitude of the measured current change can give information about the size and surface properties of the analytes at the single molecule level. A currently well-known potential application of this technology is DNA sequencing and analysis, as well as the detection of other biomolecules and nanopoarticles [1]. The use of artificial nanopores to monitor individual nanoparticles is a new biomedical sensing technique that has several advantages, such as: the use of nanoparticles as tags in biological assays, as substrates for multiplexed biological assays in solution, and as signal transducers in diagnostic assays [2].

To the goal of enable high-throughput nanopore sensing systems, we designed compact integrated instrumentation with a low input current noise at high sampling frequencies. Integrated instrumentation is required to match the pitch of the nanopore well which are on the order of 100s of microns. Due to the magnitude and the speed of the signals being measured, it is very difficult to design a system that can sample at or above 5 kHz with noise performance of <1 pA RMS, and with a capacitive load of 60 pF or above. This is because the bandwidth and input capacitance have a direct and large affect on the final measurement noise. For large arrays, the area and power utilization should be minimized.

Commercial patch-clamp amplifier systems are available, such as Molecular Devices' Axopatch 200B [3] and the

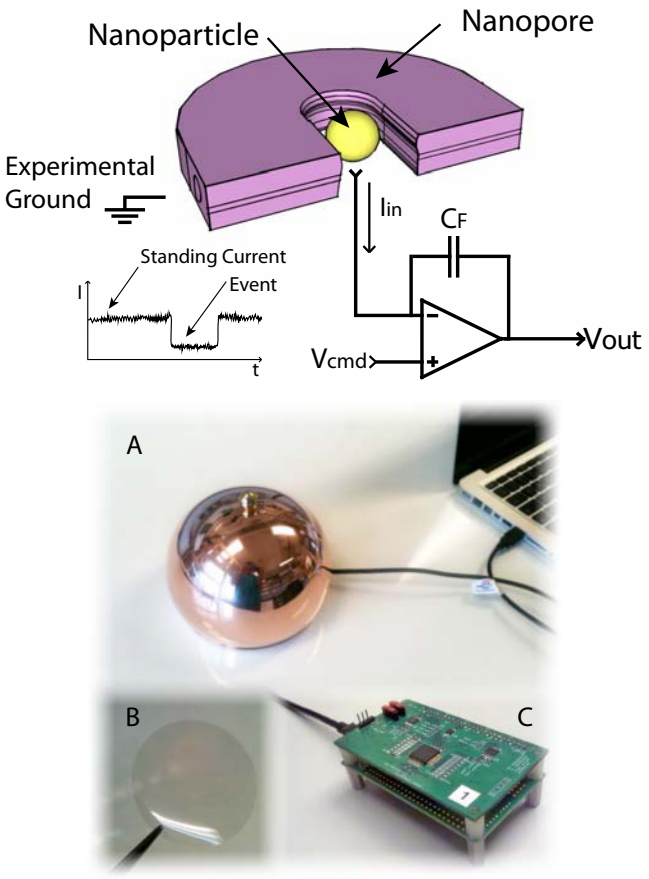


Fig. 1. Example of nanopore sensing by measuring current changes to detect when a nanoparticle blocks the pore. The system consists of (A) a spherical faraday cage to minimize environmental noise which encloses (B) a track etched nanopore and electrodes, and (C) an amplifier, and digitizer. The user controls the device and records data via USB with software running on a laptop.

HEKA EPC-8 [4], but these systems are currently bulky, high power, expensive, and not particularly suitable for simultaneous automatic measurements on a large number of nanopores in parallel, a very desirable feature in sensor arrays. A current amplifier front-end for nanobiosensors that uses a sophisticated integrator-differentiator circuit was presented in [5], but it requires a large silicon area which is less amenable to large array integration. While integrated patch-clamp systems have recently been presented such as [6] to perform voltage-

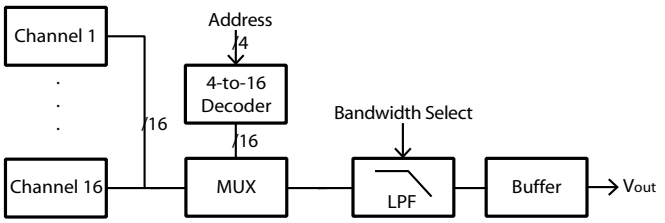


Fig. 2. System block diagram for the 16 channel nanopore amplifier system. Each channel has an individual amplifier as shown in Fig 3 which integrates and converts the input current to be measured to a voltage. Each channel is addressable and a low-pass filter and buffer are used to deliver the signal to an off-chip ADC.

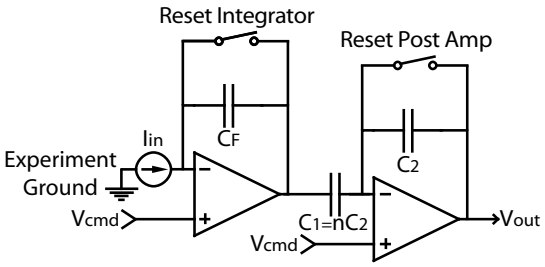


Fig. 3. One of the channels of the nanopore amplifier system as shown in Fig 2. The first stage is a current integrator with a selectable C_F of 100 fF or 1 pF and the second stage is an amplification stage that can provide an optional further gain of 5 or 10 to obtain the highest SNR while avoiding saturation based on the signal size.

clamping and current measurement, these systems consume power and area for electrode compensation circuitry which is not necessary in the detection of nanopoarticles and it is preferable to have smaller, lower power amplifiers. While there is much literature on potentiostats [7], [8], these systems work in the uA or nA level, and only achieve low noise when measuring at low frequencies, below the requirements of these applications.

In this paper we present a capacitive feedback amplifier that is suitable for integration into a single or multiple nanopore measurement system.

Since the nanopores used in this paper have a new chemical modification leading to the detection of the nanoparticles at low voltages, which to our knowledge has not yet been demonstrated elsewhere, this makes them and our amplifier particularly well suited for integration in large sensor arrays.

II. Low Current Measurement System Overview

Each channel of the system, shown in Fig. 3, features a current integrator for the first stage with selectable gain, followed by an inverting post amplifier stage. The feedback capacitance of the integrator is selectable as either 100 fF or 1 pF and the post amplifier can supply a further gain of 5 or 10 or be bypassed if it is not needed. The low-pass filter is used to filter out high frequency noise that is higher than the selected sampling frequency. This electronic system's basic design has been previously presented in [9] and is summarized in Table I, but has been modified for this application by enabling a gain of 5 in the post amp to avoid saturation when using the post amplifier, changing the integrator's reset and feedback capacitor switch to a low-leakage version for lower-noise measurements, and increasing the power and decreasing the noise of the chip's output buffer that drives the ADC. Additionally, 16 channels have been implemented, along with an output multiplexer, as shown in Fig. 2, making the chip more ideal for implementation in nanopore arrays.

To apply and fix the voltage to the nanopore under test, an amplifier with capacitive feedback is used. The overall noise of the measurement with this system will depend on the equivalent input circuit and the sampling frequency, and has previously been analyzed in [10].

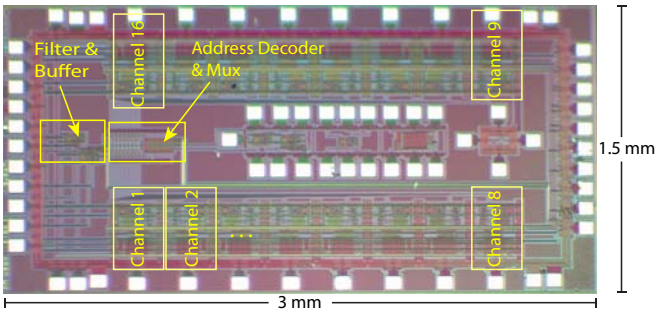
The amplifier has an output swing greater than 1.5 V to enable large currents to be monitored without requiring frequent resets. The common mode input range is 1 V, allowing for a wide range of command voltages, V_{cmd}, to be applied. The pores used in this paper have been designed to detect nanoparticles at a low voltage, so this common mode input range is sufficient.

Open Loop DC Gain	90 dB
Phase Margin	60 degrees
Gain-Bandwidth Product with 60pF load	3 MHz
Minimum Reset Time	2 μs
Input Common Mode Range	1 V
Output Swing	1.5 V
Input-Referred Noise (5 kHz bandwidth), C_F=100fF	1.35 pA RMS

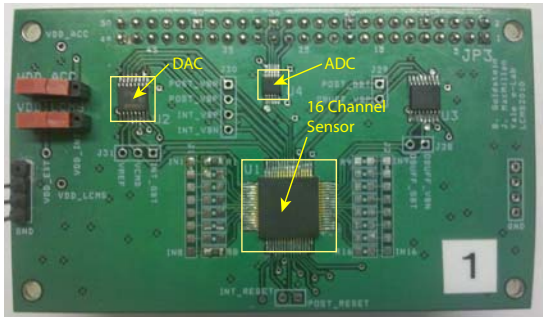
TABLE I
Summary of the Amplifier System

The input current can be determined from the sensor's output voltage by first dividing by the voltage gain of each voltage amplifying stage to determine the integrator's output voltage, and then converting this voltage to current by using the relation $I = C_F \cdot \frac{dV}{dt}$, which was performed in software. The software also performs digital correlated double sampling to reduce low-frequency noise by sampling at the start of the integration period and again before the reset.

The low current measurement system was designed and fabricated in a 0.5-μm CMOS process. The die micrograph is shown in Fig. 4(a) and occupies a total area of 1.5mm×3mm, including the bonding pads. We designed a custom 4-layer printed circuit board with separate analog and digital power planes shown in Fig. 4(b). The fabricated amplifier has 16 channels, each occupying 290 μm × 275 μm die area for the integrator, post amplifier, and capacitors. The address decoder, filter, and output buffer occupy a total of 175μ ×600 μm. The external ADC is an Analog Devices AD7685 and the system uses an Opal Kelly XEM3001v2 board which features a Xilinx Spartan-3 FPGA and a USB Controller to communicate with a PC for user configuration and data logging. The feedback capacitor, post-amplifier gain, and sampling frequency can be chosen by the user through software. The software features a custom GUI and was written in C++.



(a) Die Micrograph



(b) System Board

Fig. 4. The fabricated amplifier. Each channel is only 290×275 μm^2, while the address decoder, filter and output buffer occupy 175×600 μm^2. The total die size is 1.5×3 mm^2. It is soldered onto a custom 4 layer PCB board with supporting circuitry including a 16-bit ADC.

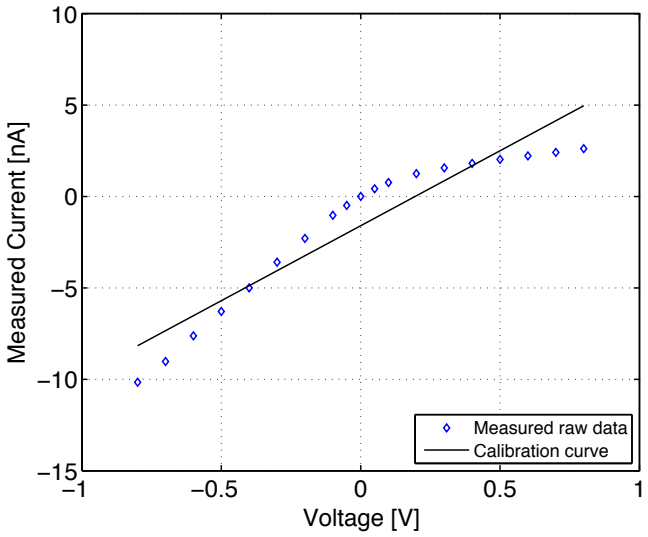


Fig. 5. Calibration curve of freshly etched nanopore in 0.1 M KCl when the ground is on the wide diameter side of the pore. The straight line is used to derive a slope value used to calculate the pore diameter. We calculated the size of the tip of the pore to be 15.66 nm.

III. Nanopore Setup

A. Fabrication of the Conical Nanopores

Polyethyleneterephthalate (PET) membranes of 12 μm thickness are irradiated at the linear accelerator UNILAC (GSI, Darmstadt) with single swift heavy ions (Pb, U) of energy 11.4 MeV/μ. An etching step is required to open the single ion impact track into a nanopore. The etching method for the fabrication of conical nanochannels in PET membranes was developed by [11]. Briefly; the heavy ion-irradiated membrane is first treated under a UV lamp, 30 minutes both sides and etched only from one side to form the conical shape of the pore. The membrane is placed between two halves of a flow cell. An etching solution (9 M NaOH) was is added on one side and the other side of the cell is filled with stopping solution (1M HCl). The etching process is carried out at room temperature. During the etching process, a potential of +1 V is applied across the membrane in order to observe the current flowing through it when the nanopore etches open. The stopping solution on the other side of the membranes neutralizes the etchant to keep the diameter of the tip small. The pore is immediately rinsed with deionized water and left in water overnight [11], [12], [13], [14], [15].

B. Characterization of the Pore Opening

After etching, the approximate diameter of the small opening of the pore (d) was estimated by using conductance measurements according to the equation $d = 4LI/\pi D\kappa V$, where L is the length of the channel which could be approximated to the thickness of the membrane, d and D are the small and large opening diameter of the channel respectively, κ is the specific conductivity of the electrolyte (0.1 M KCl), V is the voltage applied across the membrane, and I is the measured current. The conical nanochannels used in our study have a small opening (tip) diameter < 20 nm and the large opening (base) diameter of several hundreds of nanometers [13]. Both sides of a single nanopore membrane were placed in contact with 0.1 M KCl. Ag/AgCl electrodes were immersed into each solution, and a current-voltage curve was recorded for voltages between 800 and -800 mV [15]. The current-voltage data and measured calibration curve are shown in figure 5. The current trace displays a characteristic ionic correction due to the surface charge of the nanopore. The straight line is used to derive a slope value used to calculate the pore diameter.

C. Chemical Modification

Chemical surface modification of the nanopores was carried out using: N-(3-dimethylaminopropyl)-N -ethylcarbodiimide hydrochloride (98%, Fluka), N-hydroxysuccinimide (98%, Aldrich), ethylenediamine (99+%, Merck, Germany) chemicals were used as received from the supplier [12].

Activation of the carboxyl groups was carried out by immersing the membrane in 0.2 M NHS and 0.1 M EDC in aqueous solution (0.1 M MES buffer, pH 5.2) for 4 h at 4 C. After washing with distilled water, the samples were further treated with 0.1 M ethylenediamine (EDA) overnight with 0.1 M HEPES buffer (pH 8.5). Finally, they were washed two times with distilled water [12]. All reactions were carried out in the same cell used for etching.

IV. Nanopore Detection Measurement Results

The solution of nanoparticles (MPSA coated gold nanoparticle, diameter range 2-20 nm) was prepared by dissolving

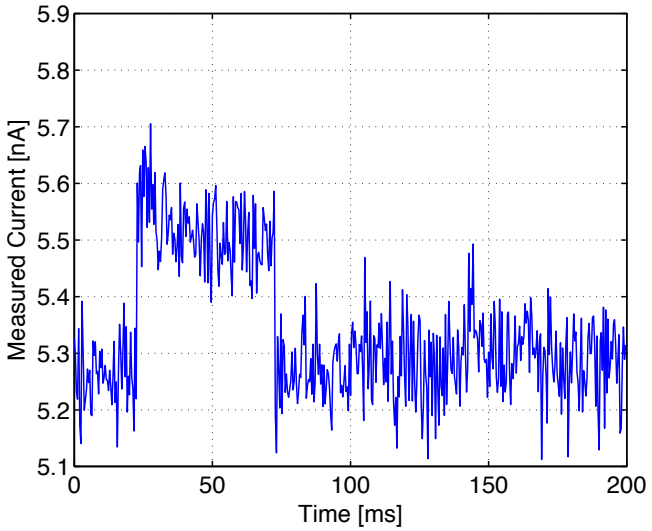


Fig. 6. Measured blocking event when negatively charged nanoparticles were directed through the positively charged pore as a result of -300 mV potential. The event starts at 22 ms and is noticeable by a 250 pA change in measured current from the baseline which lasts for 51 ms. The measurement was taken at a 5 kHz sampling rate without filtering.

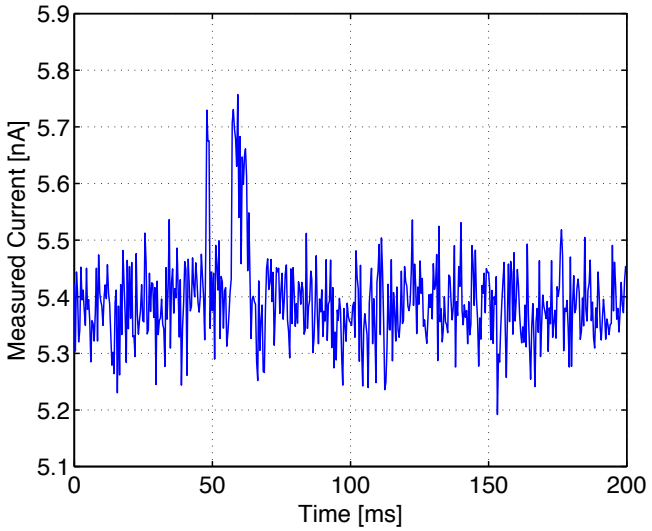


Fig. 7. By sampling at high frequency the system can catch fast events: 2 fast events are shown, each noticeable by a 350 pA change in current. The first event starts at 47 ms and lasts for 2 ms and the second starts at 56 ms and lasts for 8 ms. The measurement was taken at a 5 kHz sampling rate without filtering.

the mixture of different sizes NP (2mg/ml) in millipore water and sonicated for 10 minutes. 5 μl of this solution was added on the side of the narrow tip of the pore mounted in a Flatflow flowcell (NanoporeSolutions). -300 mV potential was applied to direct negatively charged nanoparticles through the nanopore, and current blocking events originating from interactions between the positively charged modified pore and negatively charged nanoparticles were recorded and shown in Fig. 6. Shorter events are also recorded and shown in Fig. 7. This data was taken without filtering and we believe filtering the signal will reduce the amplifier noise level by reducing aliasing.

V. Conclusion

We have demonstrated that our nanopore sensing system is capable of detecting nanoparticles interacting with the inside of specially modified nanopores, and we plan to more closely integrate the amplifier and nanopore flowcells for more accurate measurements. These pores are useful due to their ability to detect nanoparticles at low voltages.

VI. Acknowledgements

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