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Technology

A Weak Current Amperometric Technique in Physiological and Bioelectromagnetic Measurements

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Abstract. A technique for measuring ultra-low electric currents from living cells, using electrodes, biosensors or magnetic detectors is reported, based on the design of a sensitive, ultra-low-noise trans-impedance amplifier. This technique offers a low-noise, low current measurement capability down on the order of 2 x 10^{-14} amperes, with specifications such as input leakage current of less than 1 x 10^{-15} amperes and a dynamic range of 30-100 x 10^{-14} amperes. Maximum bandwidth of roughly 10KHz was observed, while working in the specified dynamic range. This set of specifications is quite satisfactory and desirable for many low-frequency applications in bioelectromagnetism and bio-amperometry. The technique finds numerous applications in studying intrinsic cellular fields and induced currents originated in cells under physiological conditions. A few applications envisaged for its possible utility include bio-sensing amperometry, general studies in bioelectromagnetism and ion transport studies in plasma membrane and mitochondrial inner membrane, by incorporation of the amplifier with suitable micro-electrodes or nano-scale electrical, magnetic or optical sensors.

Keywords: intrinsic currents, biosensors, bio-amperometry, bioelectromagnetic measurement

Introduction

A large number of important measurement applications in experimental physiology and biophysics, such as living cell bioelectromagnetism, weak current amperometry and bio-sensing with the help of embedded nanotechnology, require extremely sensitive and ultra-low-noise current measurement techniques, which can measure tiny currents at the levels of pico-amperes to femto-amperes. These measurements are typically made with micro-electrodes, quantum devices and detectors, and low-temperature cryogenic superconducting quantum interference device (SQUID) detectors, or SQUID-based nanotubes (Cleuziou *et al.*, 2006). Various electrophysiological studies with ion channels (Hamill *et al.*, 1981) or investigations on intrinsic currents (Axmacher and Miles, 2004) or weak electrical field processes within living cells (Bullock, 1997) also require sensitive instrumentation and amplification at the level of femto-amperes to pico-amperes input current. Pico-ampere amplifiers are widely available and can be easily fabricated on an electronics workbench in a physiological laboratory. Unfortunately, techniques to measure ultra-low-current (such as on the level of femto-amperes) cellular signals with least noise susceptibility are neither easy to develop nor widely available in market, in view of the special design and fabrication considerations required for their development. The main impediments faced in such designs are leakage of tiny input bias currents through the amplifier circuitry and the inherent large noise associated with measurement of ultra-low currents. With the help of special design and fabrication measures, one can venture down to an order of about 2-10 femto-amperes (with considerable noise reduction), while keeping the leakage current to a minimum extent, not affecting the measurement in a significant way. Going beyond that domain becomes an impervious task for in-house development, as at first, there are no general-purpose operational amplifiers available in market which can sense lower bias currents beyond that range, and secondly, various terrestrial and celestial effects (such as ambient electromagnetic fields in the vicinity and cosmic ray–induced electron shower events) induce tiny currents in the front-end section of the amplifier, creating unavoidable current leakages.

Industrial-grade commercial pico- and femto-ampere measurement instruments are available in market, but unfortunately they are an expensive modality and beyond the range of budgets of small biophysics and physiology laboratories,

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especially in colleges with limited budgets. Buying a commercial product off-the-shelf also precludes the experimenters from making alteration or customization in the device, in view of their specific applications. A small-scale biophysical or physiological research laboratory has to thus rely on in-house designs.

There are some public-domain designs available for low-level biological signal voltage and current amplifiers, as published extensively in literature, such as instrumentation amplifiers and pico-ampere electrometers for physiological applications, but there are a number of problems with these designs. First of all, most of the designs are for voltage mode meters. Secondly, if there are few current amplifier designs published, they are limited to nano-amperes or pico-amperes range, not presenting a design which can venture down to femto-amperes. These design ideas also pose limitations in terms of keeping the signal integrity conserved. Most importantly, these designs do not present special considerations and techniques entailed to measure ultra-low-level signals, while limiting the leakage currents and inherent noise which are coupled to small-current measurements. Thus, availability of a mere design, such as by an amplifier chip's manufacturer specifications sheet, is not sufficient to implement it in practical application.

In a biophysical study by authors to investigate the possibility and manifestations of intrinsic electric currents and endogenous electromagnetic fields in living cell systems, it was undertaken to design and fabricate a sensitive current to voltage converting amplifier which could successfully and efficiently present a practical design of an ultra-low-current and ultra-low-noise trans-impedance amplifier.

The design of this technique and its constituent amplifier differs from other conventional techniques and amplifiers in a number of ways. First of all, it presents a design paradigm which can venture down to a few tens of femto-amperes, while minimizing the leakage current and noise, with the help of its careful choice of components and special considerations followed during the fabrication of the device. Secondly, instead of just showing a way to measure the induced field currents from applied potentials, it presents a technique for low-current measurement from the living cells, which attempts to measure low-frequency intrinsic electric fields and induced femto-ampere currents, produced as a result of intrinsic effects inherent within the cell plasma membrane. This is done in an electromagnetically shielded environment and in the absence of applied fields. Third, the amplifier and measurement technique can be developed in a small laboratory with a very limited budget, costing even less than the cost of an average digital multimeter (DMM).

This technique offers an enhanced accuracy in cell amperometry or bioelectromagnetism studies by the virtue of an improvement in its meticulous design and development. Design of the amplifier is made on a special glass fiber board instead of a conventional printed circuit board (PCB) and components (including the amplifier integrated circuits) are mounted on teflon stand-offs using special considerations, as summarized in the next section. Noise susceptibility and current leakage paths are kept to a very minimum. Usage is made of some of the most precision components available in market, conserving the precision and signal integrity. The operational amplifier device used in this design, National LMP7721, has a few excellent specifications, especially the large error rejection capabilities due to the use of giga-ohm value feedback resistances, incorporated in the design. The constructed amplifier prototype is enclosed in a shielded miniature aluminum box and mounted on the micro-manipulator arm on the microscope stage, and the input of the amplifier is directly connected to the cell electrode via a short 90μ wire, without using any cable or connector. This eliminates the noise and signal losses in the cable communicating the signal from cells to the amplifier and to the data acquisition stage. Thus, adoption of special measures and extensive trials have resulted into a design which has lower current measurement range and higher accuracy and precision in terms of measurement and signal integrity, as compared to other designs described in the literature.

Materials and Methods

Amplifier design. The design of the amplifier comprises of two stages and is built around a recently-introduced precision operational amplifier IC (integrated circuit), national semiconductors LMP7721. The design reported in this paper is based on meticulous modification of a basic design as per the manufacturer's specifications (National Semiconductors, 2008). This device is an ultra-low-noise, ultra-low input bias current, operational amplifier, manufactured with metal oxide silicon (MOS) technology input stage. It has one of the lowest input bias current operations available in the market, guaranteed by manufacturer after extensive testing at around 3×10^{-15}A. Moreover, it offers superior noise performance, tested by manufacturer at 10fA-Hz$^{-1/2}$ @ 1KHz and 7nV-Hz$^{-1/2}$ @ 1KHz input-referred current and voltage noise, respectively, and a total harmonic distortion (THD) of 0.003% @ 1KHz, as claimed by the manufacturer. Its one of the most important features is curtailing of the magnitude of error produced when used with a large-value resistance, such as giga-ohms. It suppresses the large error by a factor of about 10^3 to 10^6, a great advantage in femto-ampere level current measurement using a high-value feedback resistor. This is a major advantage of

this device, which became the reason for our choosing its utility in this application, in addition to its low bias current and low noise spectral density. In addition, a theoretical (open-loop) gain bandwidth product (GBP) of 17MHz and average slow rate of around 10.0V/μs, as claimed in the manufacturer's specifications for LMP7721 in its data sheet (National Semiconductors, 2008) are appropriate for application in electrophysiology and general biophysics (although, it should be noted that, practically, the theoretical GBP described above is neither achievable in an ultra-low current amplifier design nor it is applicable in the design presented here).

Design of the amplifier is illustrated in the circuit diagram in Fig. 1. The first stage comprises a unity gain current to voltage amplifier with a zero-resistance front-end, followed by the second stage, with approximate gain of twenty inverting amplifier. The input signal is presented *via* a 50 GΩ Input Resistor to the inverting input of U1, LMP7721 device. This Resistor is only used for testing purposes to measure the minimum current readable by the amplifier. Once the amplifier is tested, this resistor is removed, enabling a direct zero-resistance connection between the input terminal and the amplifier. A precision resistor of 50 GΩ is connected as the Feedback Resistor to provide roughly 10^{10} current/voltage transfer function. However, the voltage gain of the amplifier remains unity.

Output from the current/voltage pre-amplifier is passed on to a second amplification stage, comprising of the U2 (LMP7721) *via* C3, which removes the DC voltage presented at the output of U1 from the input current. U2 has a feedback resistor, R3, which can be of any value close to around 1 MΩ (an optimal value for our application was found to be 997 KΩ), however, a resistance higher than 1.1 MΩ was found to be unsatisfactory. This stage yields a voltage gain of roughly x10 to x40 for the second amplifier (depending on the value of R3). Capacitor C5 prevents coupling of the amplifier to mains noise and also acts as a pseudo-cut-off for the high frequency content of input signal. A successive power supply filtering scheme is adopted for the amplifier power rails by means of numerous 10 nF and 100 nF capacitors at the power rail employed in the circuit. In addition, a notch filter design can also be devised at this stage for elimination of mains and high-frequency noise components. However, we did not implement it in order to conserve the original signal, as entailed in our application.

Construction. After the design, a number of prototypes of the amplifier were fabricated to achieve the optimal performance

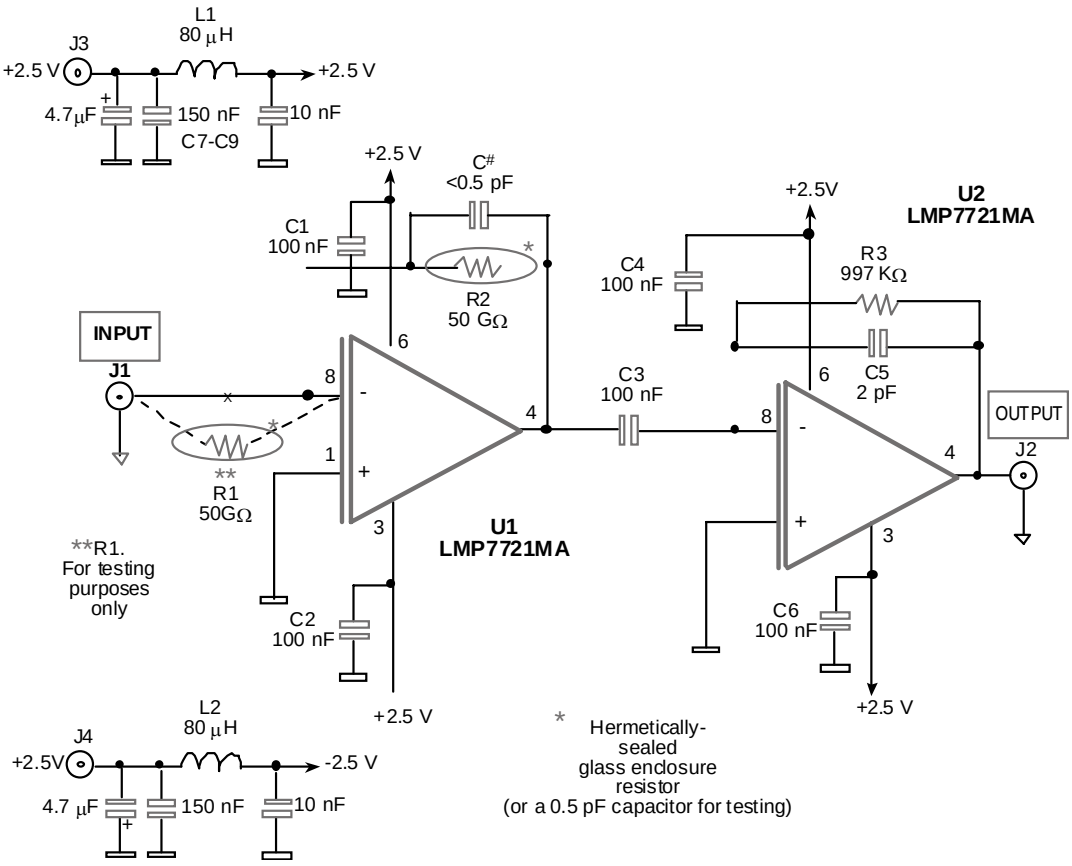

Fig. 1. A schematic diagram of the amplifier circuit.

and operation on the order of 5 to 100 femto-amperes. The device was tested with various time-domain and frequency-domain (spectrum analysis) methods used in a usual electronic workbench setup. The input current was calculated using voltage method, by utilizing Ohm's law. Alternatively, a lock-in amplifier could also be employed for this purpose. A sinusoidal low-voltage AC signal from agilent waveform generator 33220 A (Agilent, Santa Clara, CA) at various frequencies and amplitudes was applied to the amplifier's input *via* the 50 GΩ resistor. Response was recorded on Stanford SR-760 spectrum analyzer (Stanford Instruments, Stanford, CA). For instance, spectrum analyzer showed a signal of 1.000 KHz at 1.2 mV corresponding to a 1KHz, 0.1 mV input signal at the front-end of amplifier, demonstrating a total voltage gain of 12 from both stages (U1 and U2). Using Ohm's law, this input voltage, on the order of ~0.1 mV across the 50 GΩ resistor, yielded an input current of around 2 fA, which seemed to be the lowest current recorded by means of this amplifier. However, there was a substantial degradation of its performance in terms of noise. After extensive trials and re-calculation of component values and replacement by clean components, the lowest level recorded was about 20 to 30 fA, with a substantial reduction in the noise and leakage current. We take the average of this range, 25 fA, as the lowest current measured. The leakage current, after various trials and improvements on the construction of initial two prototypes, was recorded at about 1 fA. This leakage current is extremely small and impossible to be eliminated in any realistic practical design, owing to minute leakage pathways and cosmic ray shower-induced discharges. This seems to be a reasonable specification and ceiling of amplifier's capabilities, and sufficient for the measurement capabilities of the amplifier in the envisaged applications.

Significant measures were taken to minimize the current leakage and noise from the amplifier. These included, fabrication of the amplifier on a glass polyester printed circuit board (PCB), use of hermetically-sealed vacuum glass enclosure resistors (Micro-ohm Corp., Duarte, CA), suspension of the I.C. in air and contact *via* 120 µ Au-plated Cu wires enclosed in Teflon stand-offs, inverting input pin of I.C. (pin #2) and the 50 GΩ front-end resistor suspended in air with no connections to PCB and shielded with a grounded copper mesh, a grounded tight metal enclosure mounted a few centimeters from the cells sample holder, and power supply provision by batteries. A significant reduction in mains noise amplitude was observed by operation of the device in a (sufficiently) electromagnetically shielded faraday cage.

For optimal amplifier operation, it is recommended to use a regulated ±5 or 6 volts supply (even with the battery power). This power supply, based on LM7805 and LM7905 series regulators, was added to the prototype in last stages of testing, following observation of minor fluctuations in battery power (especially after prolonged burn-in hours). In addition, care must be taken not to exceed the Op-Amp's quite stringent input voltage and current limits (National Semiconductor, 2008).

For operation in the required femto-ampere dynamic range, it is extremely essential that the unit is clean and free of any deposition or contamination, especially the surface on the Op-Amp package, the Giga-ohm feedback resistor and the PCB contacts. Even traces of microscopic dust on fingertips during manual work can affect the leakage through the I.C. or these resistors. After fabrication, unit was washed with a solution of diluted ethyl alcohol (C_2H_5OH), wiped dry with high-pressure clean air and then treated in an ultrasonic bath, so as to eliminate any possible residue from the fabrication stages.

Response was also recorded and analyzed on a computer by means of a data acquisition (DAQ) system by IOtech (IOTech Corp., Cleveland, OH), using customized fast fourier transform (FFT)-based spectrum analysis routines written in national instruments labview 8.2 software (National Instruments Corp., Austin, TX).

Application in bioelectromagnetism and cellular amperometry. Amplifier design was used in the application of a bioelectromagnetism and amperometry technique devised by us to detect minute intrinsic cellular currents induced by underlying physiological processes and applied electric fields within and around the cell plasma membrane, using a budding yeast (*Saccharomyces cerevisiae*) cell model. An overview of the experiment is illustrated in Fig. 2, which is in essence a microelectrode current measurement technique. The amplifier prototype was mounted on a micro-manipulator device (Nikon, Kyoto) on an inverted microscope stage, with its input connected to a 99.9% 75 µ gold wire (Chemtel Chemicals Corp., NJ) electrode immersed in a sample of cells contained in a mini-petri dish.

A strain of wild-type *Saccharomyces cerevisiae* S288C (ATCC 26108), provided by Widger Labs at the Department of Biology and Biochemistry, the University of Houston, was preserved at 4 ºC in an autoclaved YPD agar medium. Cells were grown at a temperature of 29 ºC with agitation (160 rpm) in YPD (1% yeast extract, 2% peptone and 2% dextrose). Detailed materials and methods of growing and preparation of the cells and preparation of YPD medium are well-known (Wright and Philipsen, 1991). The main buffer used in the study was phosphate buffered saline (PBS) (Roche Corp., Indianapolis, IN). A solution was prepared with deionized water

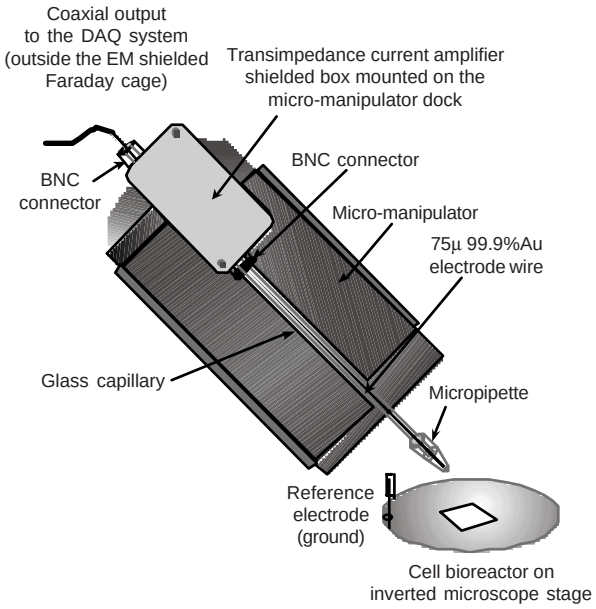

Fig. 2. An overview of the experiment to measure intrinsic electric fields as produced in cultured yeast cells as a result of various physiological processes.

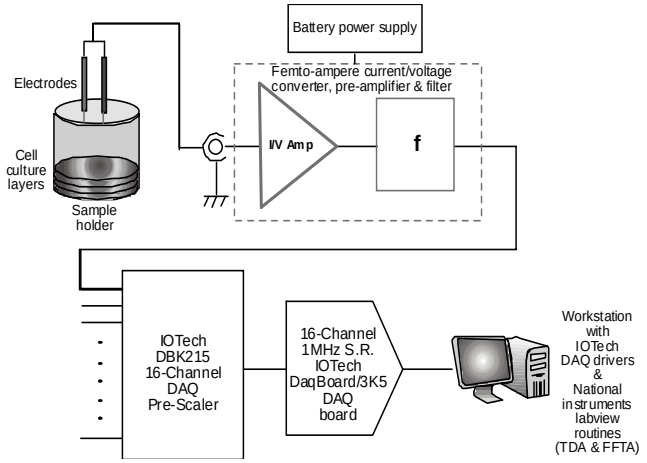

Fig. 3. A block schematic of the three-probe (two input/output electrodes and the third common ground) dielectric spectroscopy experiment to measure induced currents produced in cultured yeast cells as a result of applied time-varying electric fields. TDA stands for Time-Domain Analysis and FFTA for Fast Fourier Transform Analysis (spectrum analysis).

passed through a milliQ system（Millipore, Billerica, MA), the resistivity of which was measured at 17.8 mV-cm.

It was assured that the experiments were conducted in aerobic conditions, by means of a static aeration through an external air supply through the sides of the reactor cell vessels. Oxygen concentration in the reactor was monitored with digimed oxygen concentration monitor (Digimed, Tampa, FL). Acidity changes due to electrical fields were measured with a standard pH meter (Cole-Palmer, Vernon Hills, IL); however, no significant changes in pH were observed. The experiments were carried out at room temperature, maintained at 20.5 ^{o}C.

For intrinsic field studies, experiments involved no external electrical fields application to the cells. Signal was picked up in a shielded environment from a micropipette making contact with the cell plasma membrane. However, for measuring the external field-induced response and in the application of dielectric spectroscopy (Miller *et al.*, 2005), time-dependent electric fields of varying frequencies from 100 Hz to 10 KHz were used, by obtaining an external AC signal from the waveform generator. An external field source electrode was immersed in the cells sample holder, in the form of a three-probe mode, common ground electrode (Woodward and Kell, 1991), or four-probe mode, individual grounds (Miller *et al.*, 2005), creating a uniform electric field in the sample holder. Magnitude of the applied voltage was varied between 0.5 Vp-p to 3.0 Vp-p. Fig. 3 illustrates a block schematic of the three-probe method, designed around two main electrodes, one sensing electrode and the other applied field electrode, and one common ground.

Results and Discussion

Response was quite satisfactory as expected from the manufacturer specifications for LMP7721. Minuscule currents at the level of a few tens of femto-amperes could be measured with low noise content in our experiments. The design of the chip indeed demonstrated a conspicuous suppression in noise even in the presence of a large value resistor, yielding an RMS voltage noise at the level of a few hundred microvolts (around 250 µV on an average), unlike hundreds of millivolts using any other operational amplifier. The noise voltage and current spectral densities of the amplifier, on the order of about 10nV-Hz$^{-1/2}$ (±2n V/Hz$^{1/2}$) and 35fA-Hz$^{-1/2}$ (±5 fA/Hz$^{1/2}$), respectively, at 1.0 KHz. limit, seemed to comply well with the manufacturer-tested LMP7721 specifications of 10 fA-Hz$^{-1/2}$ @ 1 KHz and 7n V-Hz$^{-1/2}$ @ 1 KHz input-referred current and voltage noise, respectively, as reported by the manufacturer in the device data sheet. There is room for improvement and meeting manufacturer's lowest limits by improving the finesse of prototype's fabrication. There were some noise harmonics seen with floating inputs, however their amplitudes reduced conspicuously in the presence of an input signal or connection to the electrodes, as seen in the Fig. 4, which depicts recording of a 3 fA event at the input terminals (the least input bias current limit allowed by the LMP7721 device) corresponding

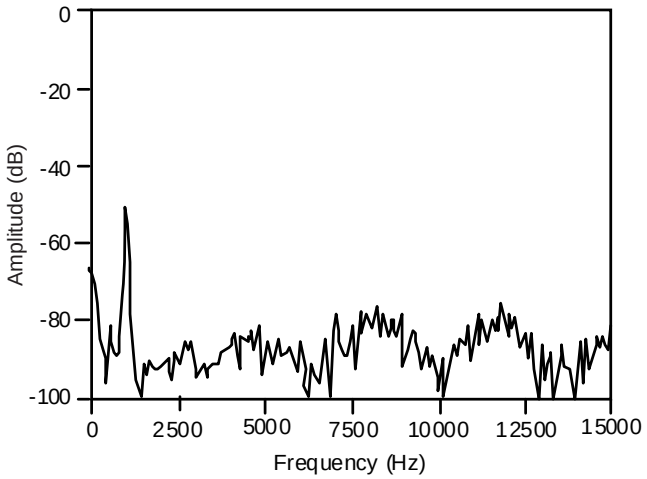

Fig. 4. Power plot of an amplified 1KHz at 0.14 mV AC test signal event as recorded by the amplifier (translating to a 3 fA input current recording).

to a 0.14 mV, 1 kHz input signal. But at this level, there was a large noise component in the signal. After extensive trials, a reasonable performance, in terms of the least noise, was obtained at an input current level of average 25 fA, which we claim as the lowest current limit of measured input current, using this amplifier.

The bandwidth of amplifier seemed to be satisfactory till 9.8 KHz before a substantial signal-to-noise ratio degradation is observed. After further development of the amplifier, this limitation on frequency range could be improved.

The transfer function or the trans-impedance gain of the front-end amplifier is calculated using the relationship of output voltage to input current in an inverting amplifier, as follows:

$$V_{out} = - I_{in} \times R_F \quad \text{.....(1)}$$

With an input current of 30 fA and a feedback resistor value of 50 GΩ, as used in this design, a value of 1.5 mV output voltage is determined. This seems to be the maximum magnitude of the obtained voltage, with low noise susceptibility, corresponding to a low-current on the order of few tens of femto-amperes which can be measured with the front-end stage. After signal conditioning through the second-stage amplifier, this is amplified about ten-fold and one can obtain few tens of milli-volts from the constructed prototype while measuring an ultra-low-current. Typically, the range of obtained voltage amplified from a cellular intrinsic signal is 0-20 mV, corresponding to minuscule currents on the order of 1-35 fA being generated within the cell plasma membrane.

The transfer ratio for the two-stage amplifier with 15 mV measured output and 30 fA input current was determined to be 0.05×10^{12} rho (or 0.05 pico-rho) using the relationship for transfer ratio:

$$k = \frac{V_{out}}{I_{in}} \quad \text{.....(2)}$$

This transfer ratio, expressed in terms of the dimensions of resistivity, is found to be quite high as expected, and complies to the transfer function of the amplifier. The magnitude of this ratio, in the dimensions of resistivity, is slightly less than the resistivity of rubber and glass (Serway, 1998).

This implies that, if the minimum possible detected current, distinguishable from noise, or the minimum detected signal (MDS), of this amplifier is around 30 fA, the input electronic transduction capacity of the amplifier and this technique is to transduce an electronic pulse of approximately 18,720 electrons/second (from the fact that 1 fA current involves transport of 6242 electrons/second) with the help of a suitable input sensor.

A number of three successive prototypes were built, beginning with the use of another low bias current amplifier (LMC662) and a set of input and feedback resistors of 10 GΩ and utilizing various configurations up to 100 GΩ, before a successful operation could be achieved at the input range of around 5 fA-25 fA with 50 GΩ, using LMP7721. A use of resistors above this optimal value of 50 GΩ, such as 100 GΩ, was found to be ineffective in decreasing the input current while keeping the noise to a minimum value. It was found that large value resistors (higher than 50 GΩ) are inherent with large noise susceptibility and low SNR, as described earlier. The best value measured is thus determined at a range of 20-30 fA, with minimum possible leakage current and high SNR.

After testing of the amplifier with spectrum analysis, it was used in a micro-electrode-based biological experiment, incorporating a bioreactor and patch-clamp design, using gold and gold-plated-tungsten (Au-W) micro-electrodes. Aim of this experiment was to investigate the form and manifestations of tiny currents and intrinsic noise produced by metabolic pathways or physiological processes in living cells and organelles primarily in cultured wild-type *Saccharomyces cerevisiae* (brewer's yeast) cells *in vitro*. Cell membranes have been known to be associated with membrane noise (Verveen and DeFelice, 1981) and their intrinsic ability to amplify external electric fields. The membrane noise is beyond the thermal or white noise and in fact some form of stochastic cellular activity, which has been termed to carry a valuable signature of underlying physiological processes, a possibility cited by Bullock in his detailed treatise (Bullock, 1997) in the realm of neurophysiology. The technique revealed presence of a simi-

lar intrinsic noise from respiring and active yeast cells. Amplitudes of aerobic living cells were found to be conspicuously higher (about ~70%) than dead or anaerobic cells or the phosphate buffered saline (PBS) medium alone without cells. We measured currents ranging from 20 fA to 55 fA from the active, respiring yeast cell membranes, induced as a result of a number of processes, without any applied or ambient electric fields. The source and underlying mechanisms of these currents, which are referred as intrinsic noise, may be ion channel transport across the membrane or some correlations with the metabolic states of the cells. These possibilities are currently being investigated and a few of our reports in this context are in the process of publication. Fig. 5 illustrates the sample intrinsic electric response (amplified hundred times in this figure) from the plasma membrane of yeast cell while in its active aerobic-respiring state and under physiological conditions, as measured with this technique, and in the absence of any ambient or applied external electromagnetic fields. Fig. 6 depicts a dielectric response (most likely from the cell plasma membrane in view of very low frequency), from an active and aerobically respiring yeast cell, in response to an applied AC field of frequency 1.2 KHz @ 1.0 $V_{p\text{-}p}$, using a three-probe configuration. The effects of medium in which cells were suspended have been subtracted from the amplitude displayed.

One of the final amplifier prototypes, as used in testing, is illustrated with the help of a photograph in Fig. 7, as assembled in the Biophysics Research Laboratory at the Texas Center for Superconductivity at University of Houston (TcSUH). Some of the special considerations entailed for manufacturing such amplifiers can be appreciated from the photograph.

While testing of the amplifier, it was revealed that amplifier is not only very sensitive to surrounding electrical and magnetic fields, but also to external vibrations present in environment, such as acoustic vibrations from the surroundings. Electromagnetic and acoustic shielding presented themselves as mandatory requirement for the reliable measurements using this device.

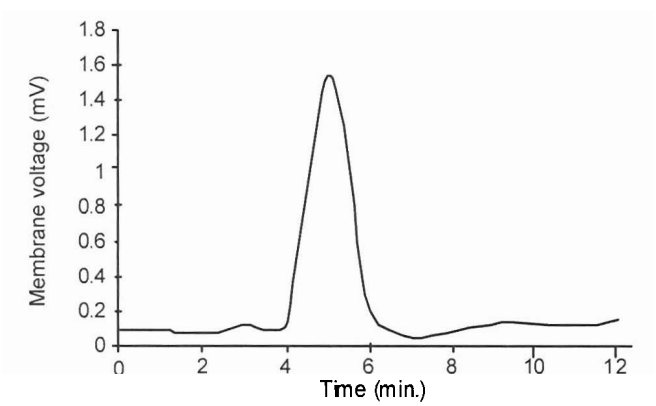

Fig. 5. A sample intrinsic cellular signal as measured *in vitro* from cell membrane of an aerobically-respiring yeast cell (x100).

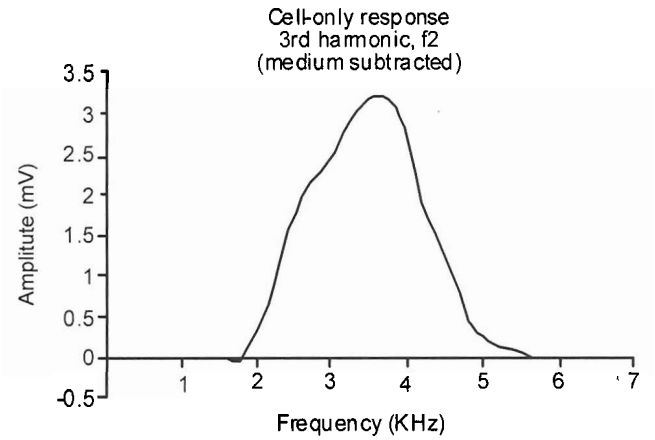

Fig. 6. A snapshot of an induced AC signal as measured from respiring yeast cells in response to an applied AC field, using three-probe method.

It is difficult to make an accurate claim for the most minimum possible current level measured by the amplifier, in view of the fact that every operational amplifier is susceptible to parasitic capacitances, which affect the measurement of input current using the Ohm's law-based voltage method (by measuring the voltage across the 50 GΩ resistor, as done in this study). However, the operational amplifier integrated circuit employed in this design, national semiconductor LMP7721, guarantees a tested ultra-low-level femto-ampere operation at very low noise voltage and current densities, as reported in the abstract. Nevertheless, we have done earnest efforts in meeting the lowest possible limit of measured current, while keeping signal integrity conserved. It can be surmised that if contin-

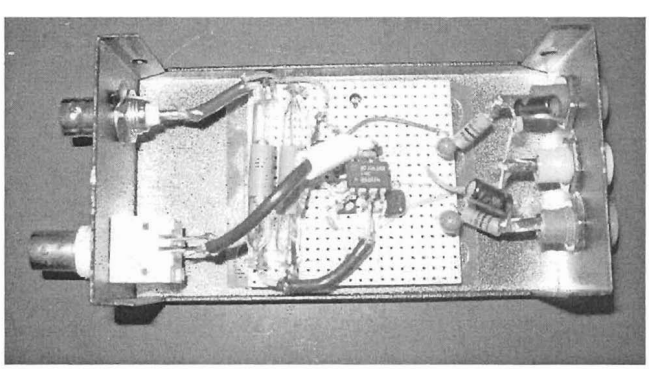

Fig. 7. A photograph of amplifier prototype, where the fiberglass PCB, the amplifier chip and high-resistance vacuum-sealed resistors, can be clearly seen.

ued improvements are made in the proper selection of components and careful assembling of the amplifier, as described in this report, it could possibly result into an amplifier capable of measuring a lowest possible current of 1 to 3 fA. Nevertheless, in our study we could make measurements with currents at the scale of around 25-30 fA at a bandwidth of 10 KHz, with satisfactory noise suppression. This is our claimed minimum input level of measurement, which is a reasonable lower limit and sufficient for a broad spectrum of applications in biophysics and electrophysiology.

Although, theoretically the claimed bandwidth for LMP7721 is quite high, as highlighted earlier, but our analysis of the amplifier and testing revealed the experimental bandwidth limited to around 10 KHz. This constrains the amplifier's application to its use limited to low-frequency signals regime, such as in general cell electrophysiology, low-frequency biological amperometry, electroencephalography (EEG) and electrocardiography (ECG) etc. Modifying the design of the first stage front-end amplifier can increase the bandwidth window manifold, but would adversely affect the minimum current measurement capabilities and noise performance. A trade-off would be required in the two parameters, depending on the problem at hand.

Conclusion

Design and development of a very sensitive, low-noise and low-cost amplifier have been carried out, as reported in this paper, which is found to work at an input current dynamic range of ~2 x 10^{-14} to 1 x 10^{-13} amperes, with a lowest measured current limit of around 25 femto-amperes with low-noise content, yielding voltage and current noise spectral densities on the order of about 10n V-$Hz^{-1/2}$ and 35 fA-$Hz^{-1/2}$, respectively, at 1.0 KHz. Initial testing was done with workbench time-domain and spectrum analysis methods. The amplifier was incorporated in an application study by investigating its response to very low current sources, such as membrane currents and intrinsic noise in cultured living cells, as well as in recordings of minute changes in the harmonic response of cells to applied sinusoidal electrical fields. On the basis of this, a technique was developed to measure ultra-low currents within the living cell plasma membranes.

Another application of this amplifier and its based technique lies in the experimental measurement of mass-transport-diffusion current (Taylor and Schultz, 1996) in biosensors or bioelectronic electrodes which work on the principle of amperometric transduction of biological processes. By use of a suitable biosensor, such as a carbon electrode, or an embedded field effect transistor (FET) in a constant-potential configuration, minuscule fluctuations in current may be detected which are the direct measure of the rate of electron transfer reaction in the diffusion layer (the region of solution in bioreactor in which the sensor/electrode is immersed). The technique can be utilized in measuring the electron transport current, which has a mathematical value as expressed by Equation 3.

$$I = \frac{nFADC}{\delta} \quad \text{.....(3)}$$

Where F is the Faraday's Constant, A the area of electrode, δ the thickness of diffusion layer, C concentration, D the diffusion coefficient and n the number of transferred electrons.

Knowing the current, one can easily deduce the diffusion coefficient in a biological experiment. With the incorporation of this amplifier design, transduction of a weak stream of mass-transport-diffusion electrons from a biosensor can be detected, which would otherwise be difficult with conventional amperometry techniques. This area needs investigation. An experiment in this direction is currently being considered by us for carrying out.

The design has great potential in its application in many areas in general biological and physiological measurement. It is earnestly hoped that this design will stimulate further efforts in this direction which could bring forth improved designs of similar ultra-low-level-current, ultra-low-noise and low-cost amplifiers, advancing the field of measurement science and technology in biosensors, bioelectronics, electrophysiology and quantum computing applications. By offering a measurement technique to measure non-thermal noise stochastic signals, which may be rich in knowledge pertaining to underlying physiological processes, as suggested by Bullock (1997) in the case of neurophysiology, this technique has great potential to be further investigated.

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