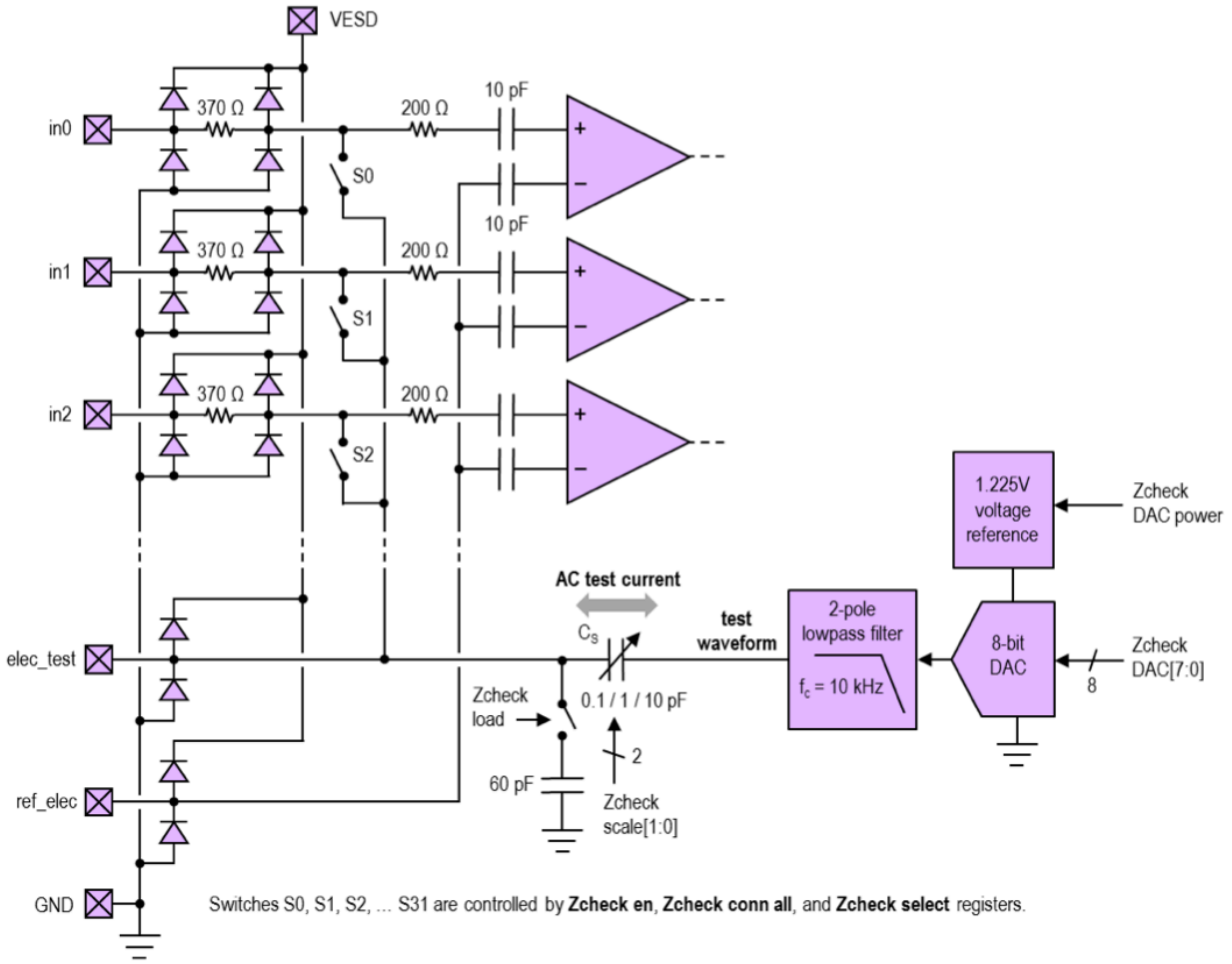
# Main Functionality

- Impedance check (1kHz or 1 - 10kHz), Test Target Impedance Range: 0.1k, 100k, 1M
- Electroplating
  - Ideally, a 3-electrode setup: counter electrode, reference electrode, and working electrode (electrode site)
  - Constant-current-based driver: 0.1n - 1mA?
- Megasonic cleaning
  - MHz piezoelectric ceramic
  - Cup autoclave compatible (121C)
- UVC LED sterilizer




Switches S0, S1, S2, … S31 are controlled by **Zcheck en**, **Zcheck conn all**, and **Zcheck select** registers.

## Electrode Impedance Test

All RHD2000 chips have built-in circuitry that provides selectable, direct access to any of the amplifier input pins for the purpose of measuring the impedance of electrodes connected to the chip. Additional on-chip circuitry is provided to generate an AC current waveform needed to measure electrode impedance. Also, an input pin (**elec_test**) is provided for connecting external current or voltage generators to any selected amplifier input pin.

The figure on the next page shows a detailed schematic of the amplifier array input circuitry on the RHD2132; input circuitry for the RHD2216 is similar. Transistor switches S0 through S31 can be closed to connect one selected amplifier to the on-chip current generator as well as the auxiliary input pin **elec_test**. If the register **Zcheck en** is set to zero, all switches remain open. This is the normal mode of operation for the chip.

If **Zcheck en** is set to one, then the switch corresponding to the amplifier that is selected by the **Zcheck select** register is closed, and that amplifier's input is connected to the on-chip current generator and the **elec_test** pin. This mode of operation should be used for measuring the impedance of individual electrodes. If an AC current waveform (with no DC current component) is generated on chip or applied to the **elec_test** pin from an external source, then the resulting voltage waveform will pass through an amplifier and may be observed by the ADC. The impedance of the electrode may then be calculated as the ratio of peak voltage to peak current.

Note that this technique requires small currents, as the RHD2000 amplifiers saturate for input voltages larger than ±5.0 mV. For example, a 5 nA peak current will elicit a 5 mV peak voltage with an electrode impedance of 1 MΩ.

Note that any impedance measurement will include the capacitance of the on-chip amplifiers (10 pF), the ESD protection diodes (0.4 pF), and approximately 1.6 pF of parasitic capacitance associated with the bond pad and QFN package. This 12 pF of capacitance has an impedance magnitude of 13 MΩ at 1 kHz, and should only affect impedance measurements for relatively high-impedance electrodes. The ESD protection resistors have very small values, and are unlikely to significantly affect impedance measurements of typical electrodes.

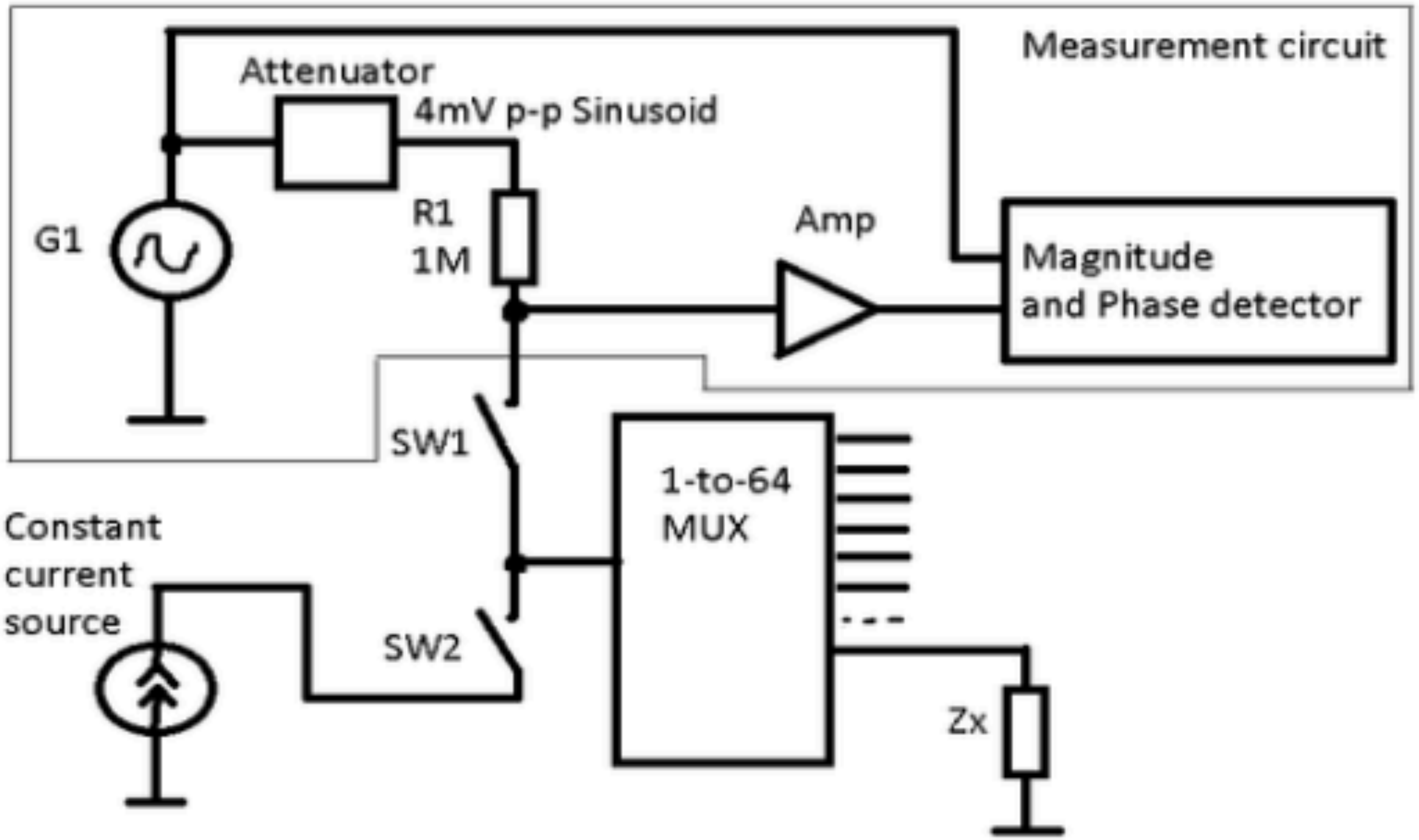
The RHD2216 has two independent input pins for each amplifier, so an extra register called **Zcheck pol sel** is provided to select between the positive and negative amplifier input pins during electrode impedance testing. When **Zcheck pol sel** is set to zero, **Zcheck en** connects the on-chip current generator and **elec_test** to the positive input terminal (**inX+**) of the selected amplifier. When **Zcheck pol sel** is set to one, the impedance testing circuitry is connected to the negative input terminal (**inX–**).

## Electrode Activation

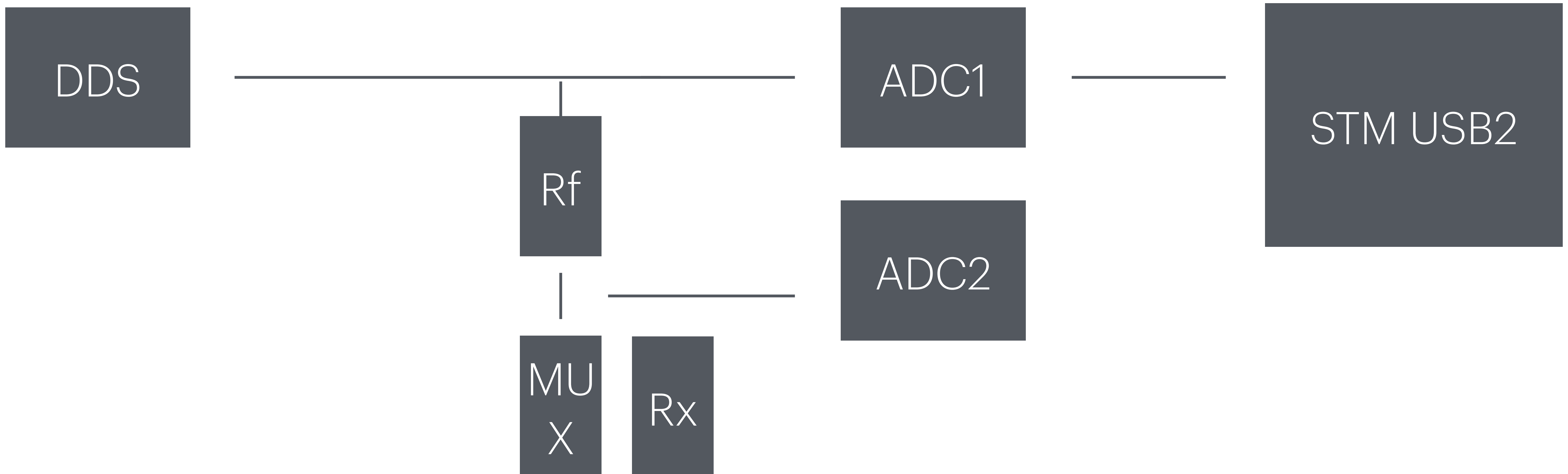
The on-chip switches S0 through S31 may also be used to apply DC voltages to selected amplifier input pins in order to activate or electroplate various types of electrodes after they have been connected to the chip. DC voltages must be applied through the **elec_test** pin. During this process, **VESD** will need to be connected *temporarily* to a higher voltage (such as $V_{DD}$) to prevent forward biasing of the ESD protection diodes. Note that the "on" resistance of each on-chip transistor switch (nominally around 400 Ω) increases significantly as the applied DC voltage rises above ground.

If negative voltages need to be applied to the electrodes (relative to some electrolyte) then the electrolyte must temporarily be held at a potential above chip ground. The DC voltage applied to **elec_test** must remain between chip ground and **VESD** at all times. See the Intan Technologies RHA2000 Series datasheet for more information on electrode activation and electroplating. The concepts described there can be applied to the RHD2000 chips.

If both the **Zcheck en** and **Zcheck conn all** bits of Register 5 are set to one, all switches are closed simultaneously. This connects all amplifier inputs to the **elec_test** pin in parallel. This mode of connectivity may be used to activate or electroplate all electrodes in an array concurrently. This mode *cannot* be used to test the impedance of all



Measurement circuit
Attenuator
4mV p-p Sinusoid
G1
R1
1M
Amp
Magnitude
and Phase detector
SW1
1-to-64
MUX
Constant
current
source
SW2
Zx

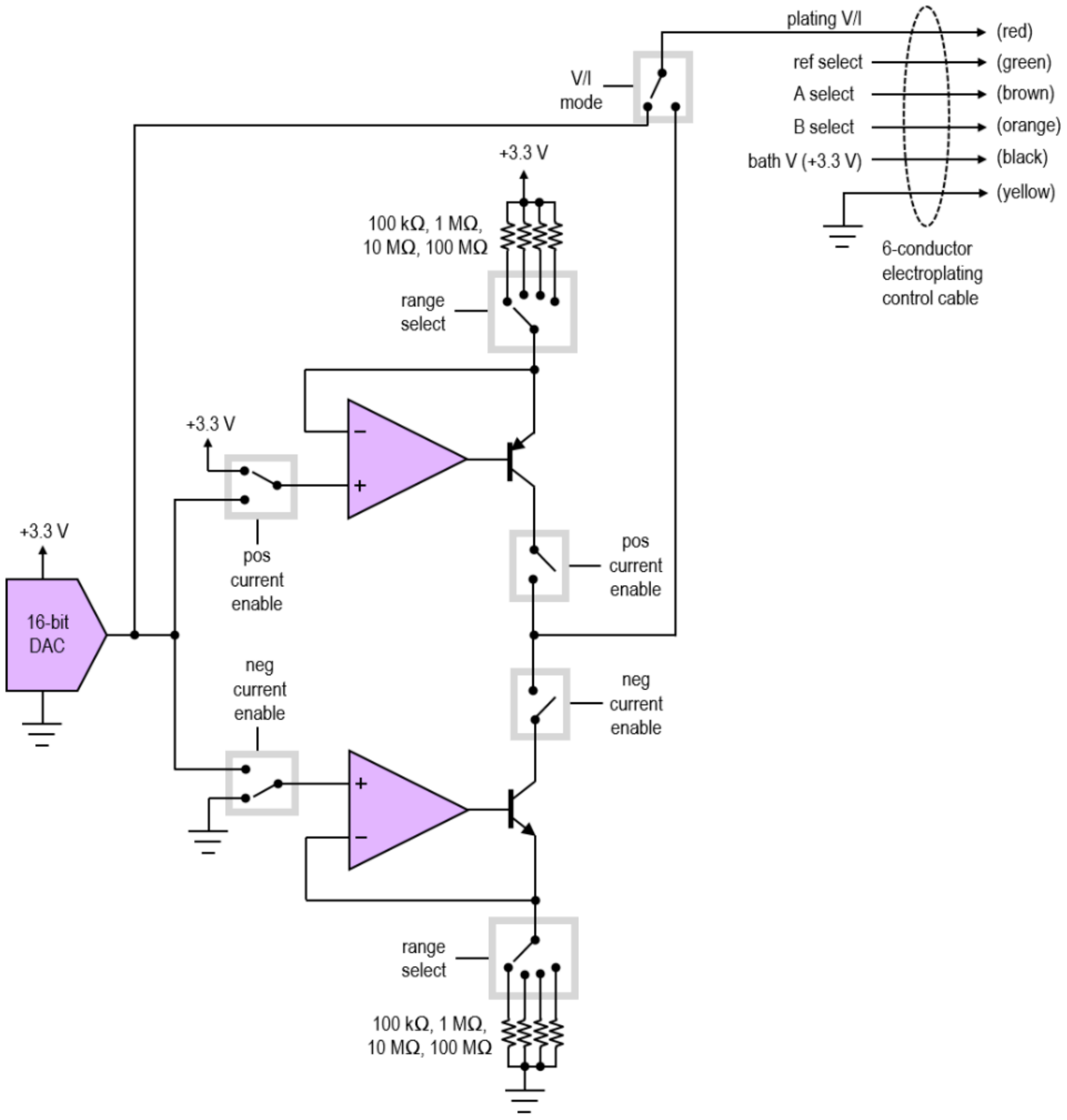



DDS
ADC1
STM USB2
Rf
ADC2
MU
X
Rx

# Circuit Design and Operation

This section describes the hardware on the RHD2000 electroplating board that generates plating voltages and currents. It is not necessary to understand this material in order to use the system with the MATLAB GUI software provided. This information is provided for advanced users who wish to modify the software or develop similar hardware.

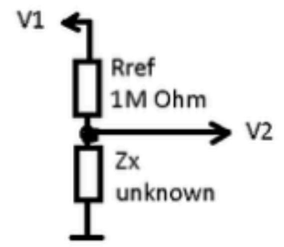
The schematic diagram below shows a simplified circuit schematic of the hardware on the electroplating board. Switches surrounded by grey boxes are digitally controlled CMOS switches (74HC4052 and 74HC4053 integrated circuits). The amplifiers are OPA4170 rail-to-rail operational amplifiers well-suited for operating from the relatively low (3.3 V) voltage supply. The NPN bipolar transistor is a BC107 and the PNP bipolar transistor is a 2N3964; both of these are general-purpose devices. The DAC is an Analog Devices AD5662 – the same DAC used in the RHD2000 USB interface board. This DAC can produce an output voltage between 0 and +3.3 V.






## Appendix A: Principle of Operation

For measuring impedance, the nanoZ utilizes a voltage divider circuit:



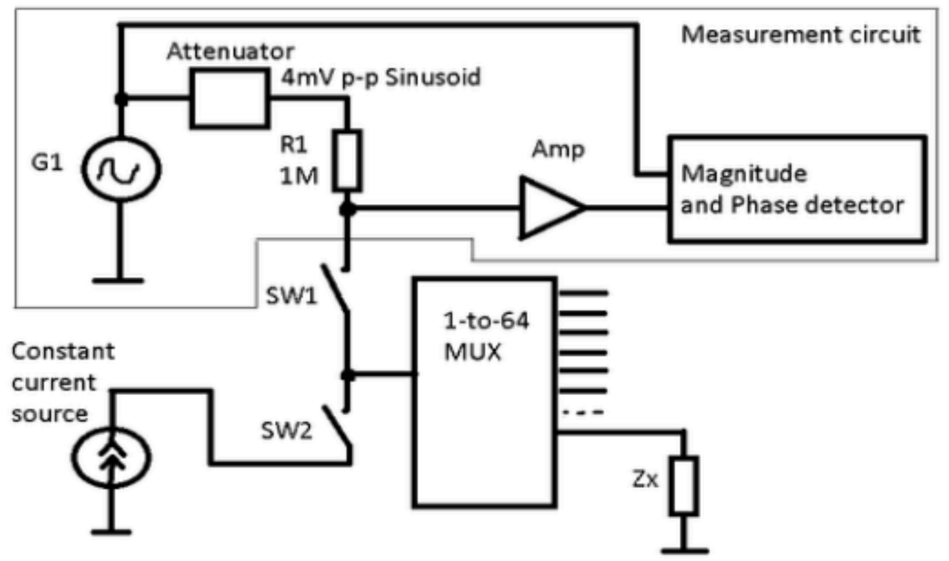

According to Ohm's law, the ratio of voltages $V1$ and $V2$ in the circuit is:

$$\frac{V1}{V2} = 1 + \frac{R_{ref}}{Z}$$

This formula generalizes to AC sinusoidal signals where $V1$, $V2$ and $Z_x$ are complex numbers whose angles represent phase relations in the circuit. When a known voltage $V1$ is applied, and $V2$ is measured, it is possible to solve the above equation for $Z_x$, which is exactly how the nanoZ measures impedance.

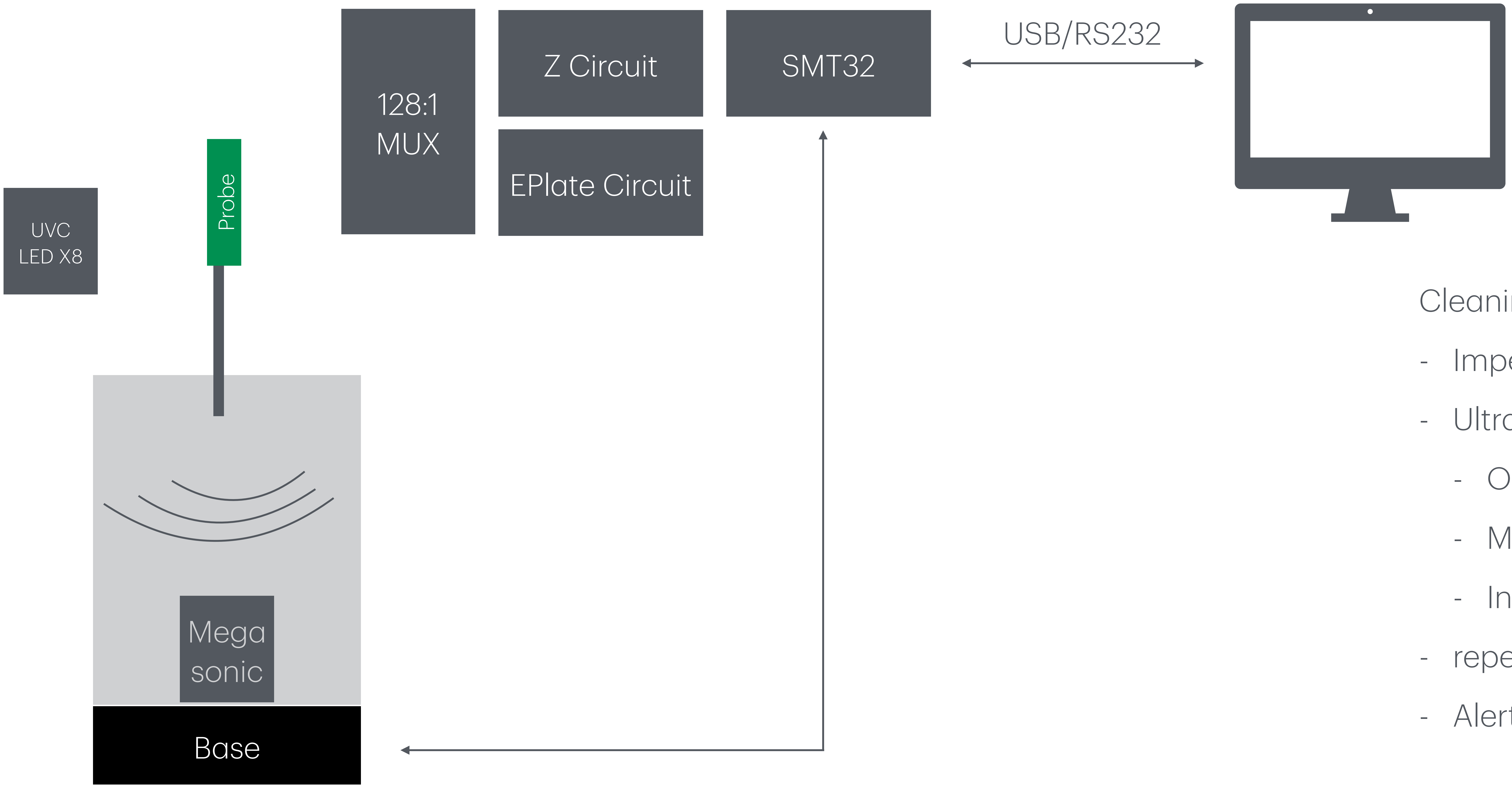
During impedance measurement test currents flow through the circuit. The nanoZ uses a 4mV peak-to-peak sinusoidal waveform for $V1$, which yields a maximum test current through $Z_x$ of 1.4nA RMS when $Z_x$ is approaching zero, and 0.7nA RMS when $Z_x$ is 1MOhm.

The nanoZ has a single measurement circuit, including the generator voltage $V1$, the amplifier for $V2$, and the reference resistor $R_{ref}$. Different channels, having different electrode impedances $Z_x$, are connected to this circuit via an on-board 64-to-1 analog multiplexer. Here is a simplified schematic of the overall circuit:



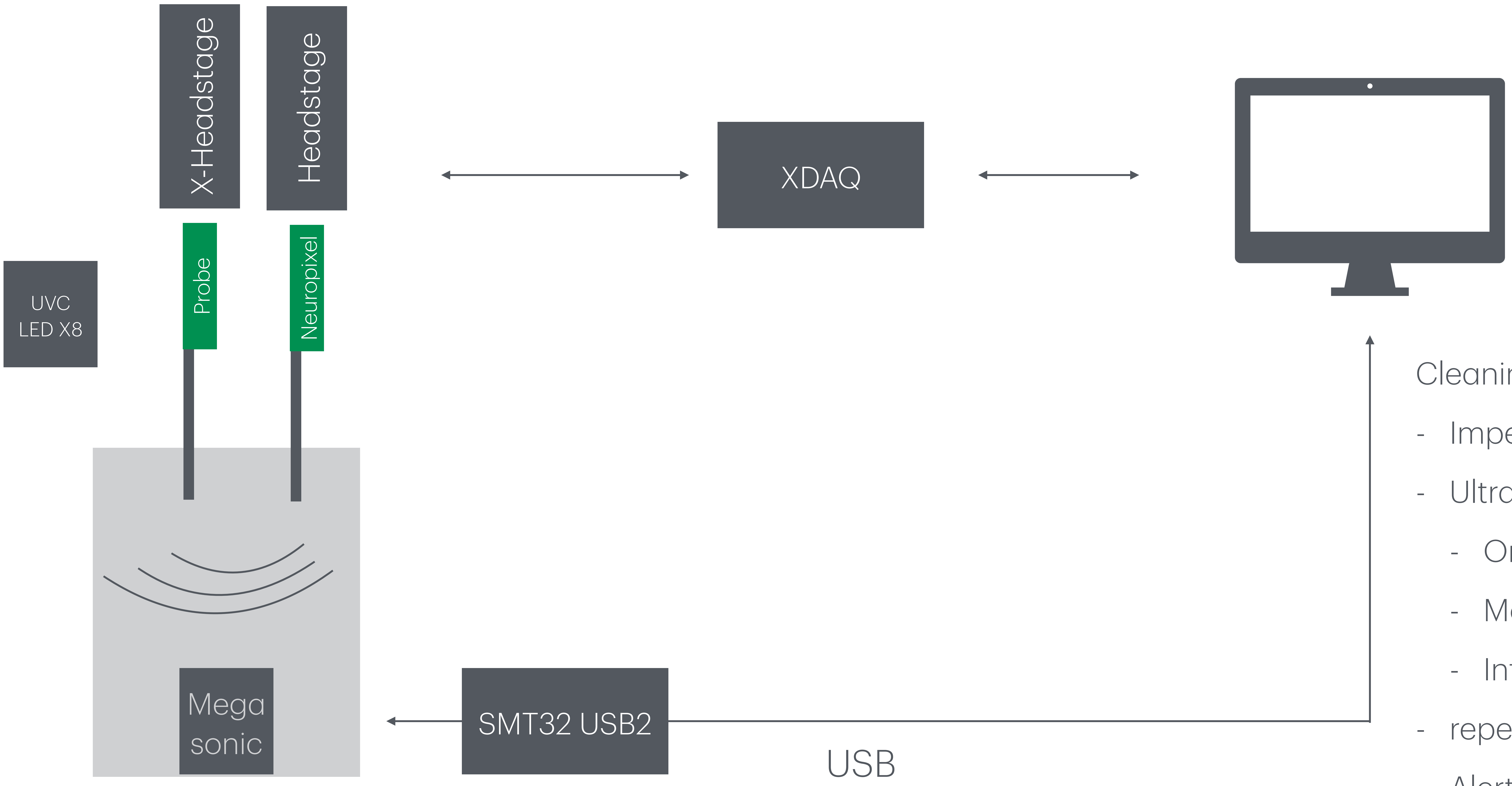

Either the impedance measurement circuit or the electroplating constant current source can be connected to a channel via switches SW1 and SW2.

The electroplating current source is programmed by a voltage coming from an 8-bit DAC, yielding 256 current steps between -12uA (electrode negative) and +12uA (electrode positive). The DAC can produce both DC and alternating waveforms from the nanoZ's on-board memory.




Cleaning Protocol

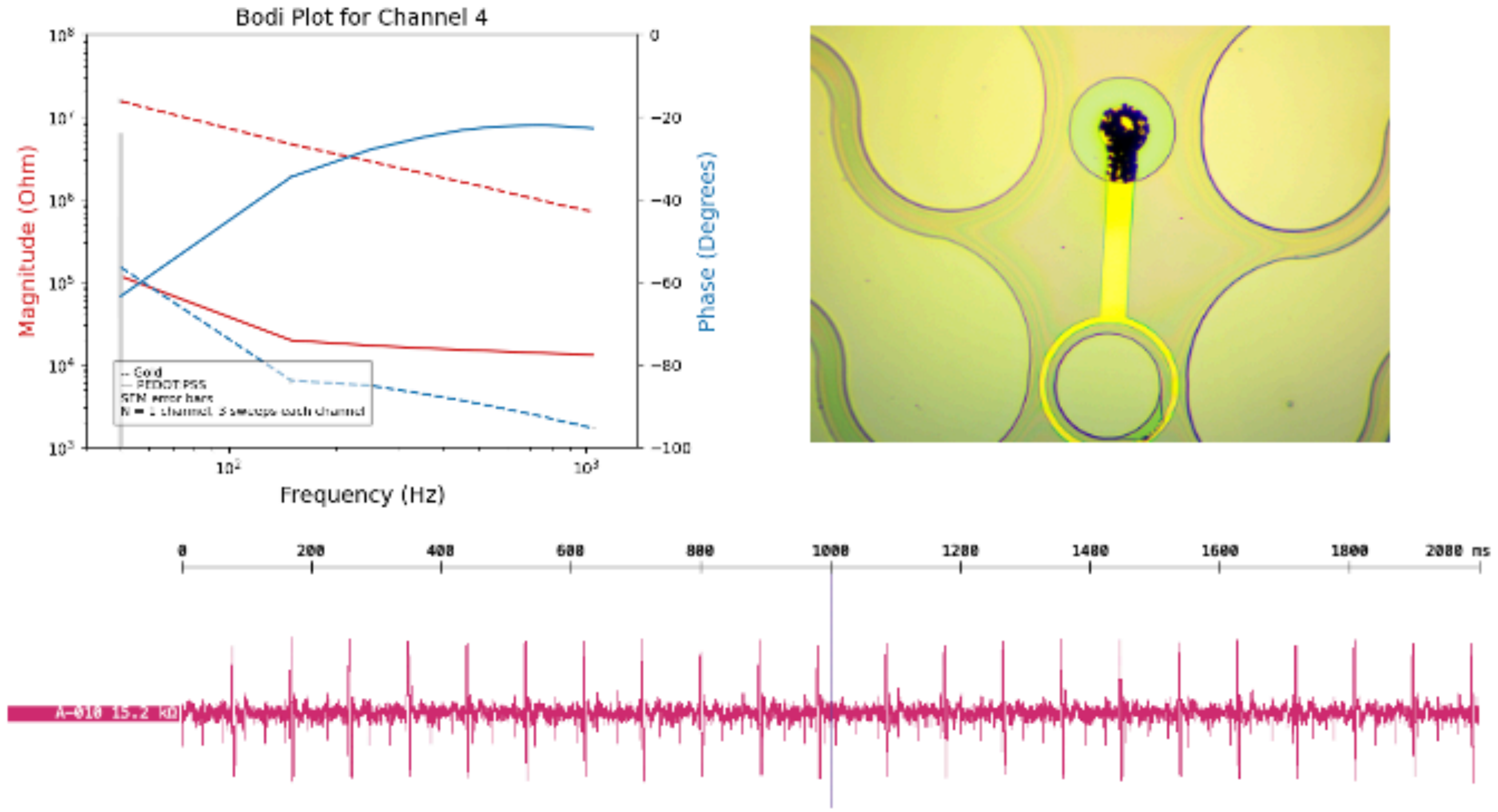
- Impedance check
- Ultrasound
  - On-Off
  - Modulation
  - Intensity
- repeat, log Z
- Alert Complete



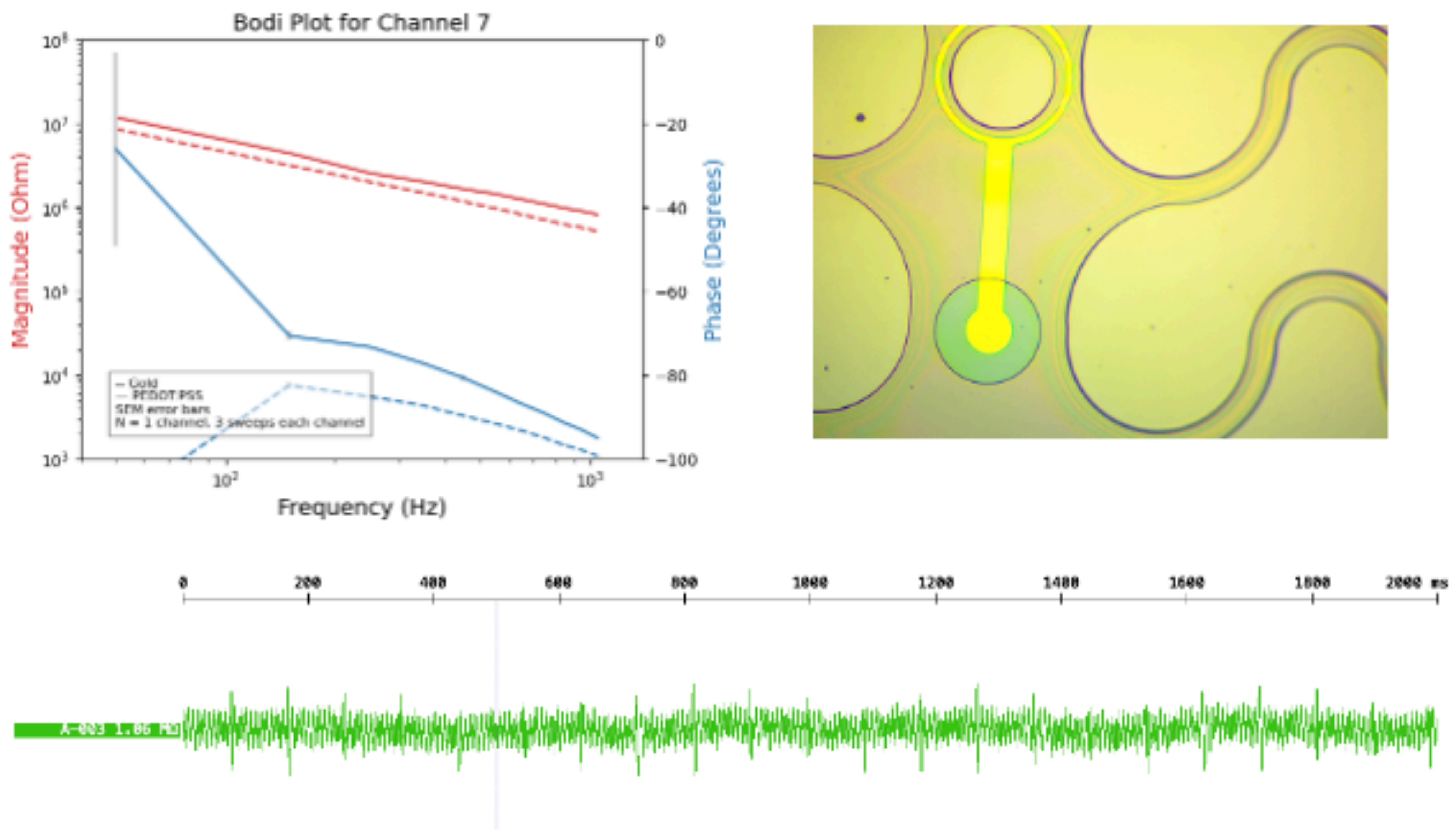

Cleaning Protocol

- Impedance check
- Ultrasound
    - On-Off
    - Modulation
    - Intensity
- repeat, log Z
- Alert Complete

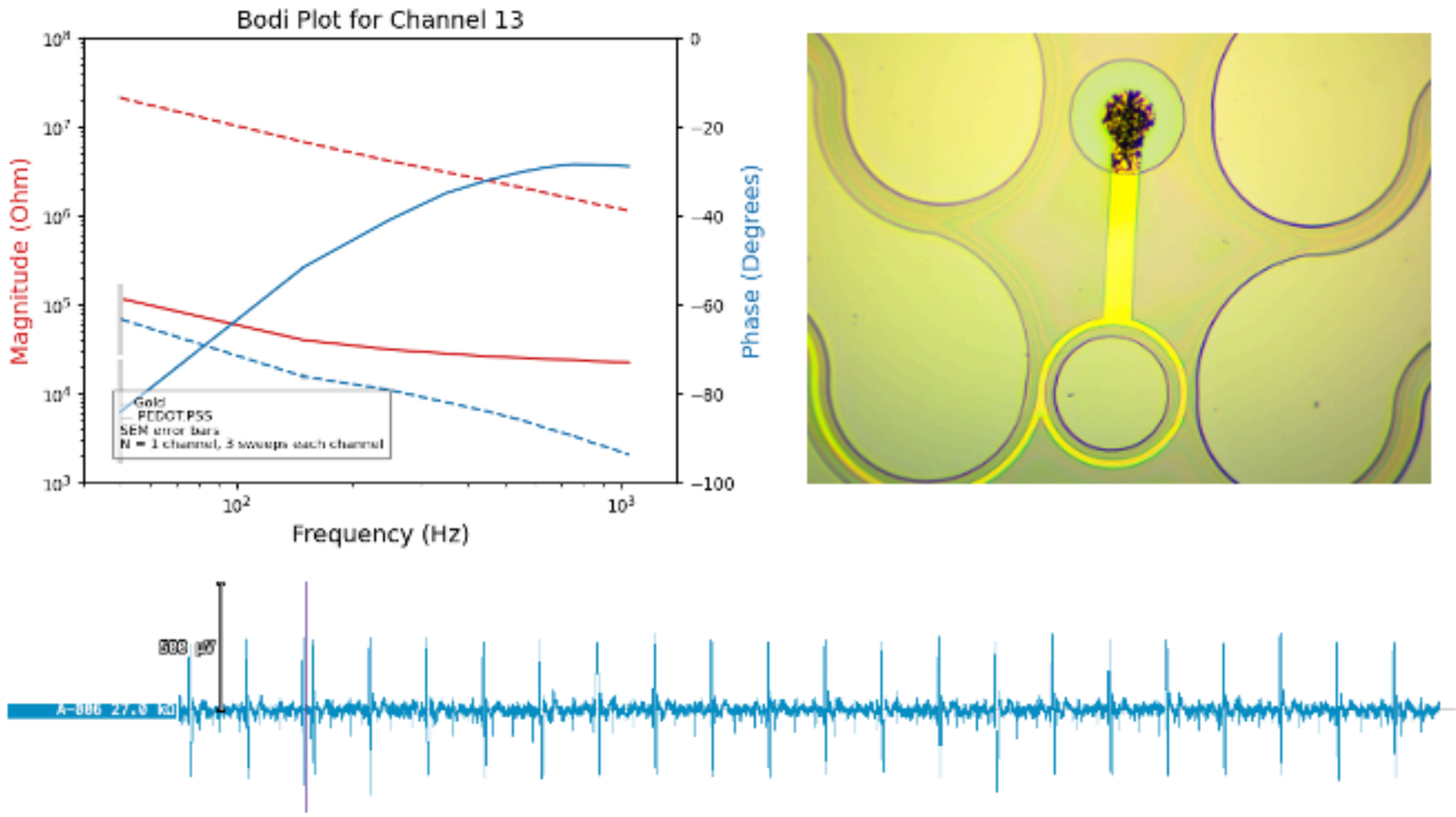
# 10 nA - 10 Min



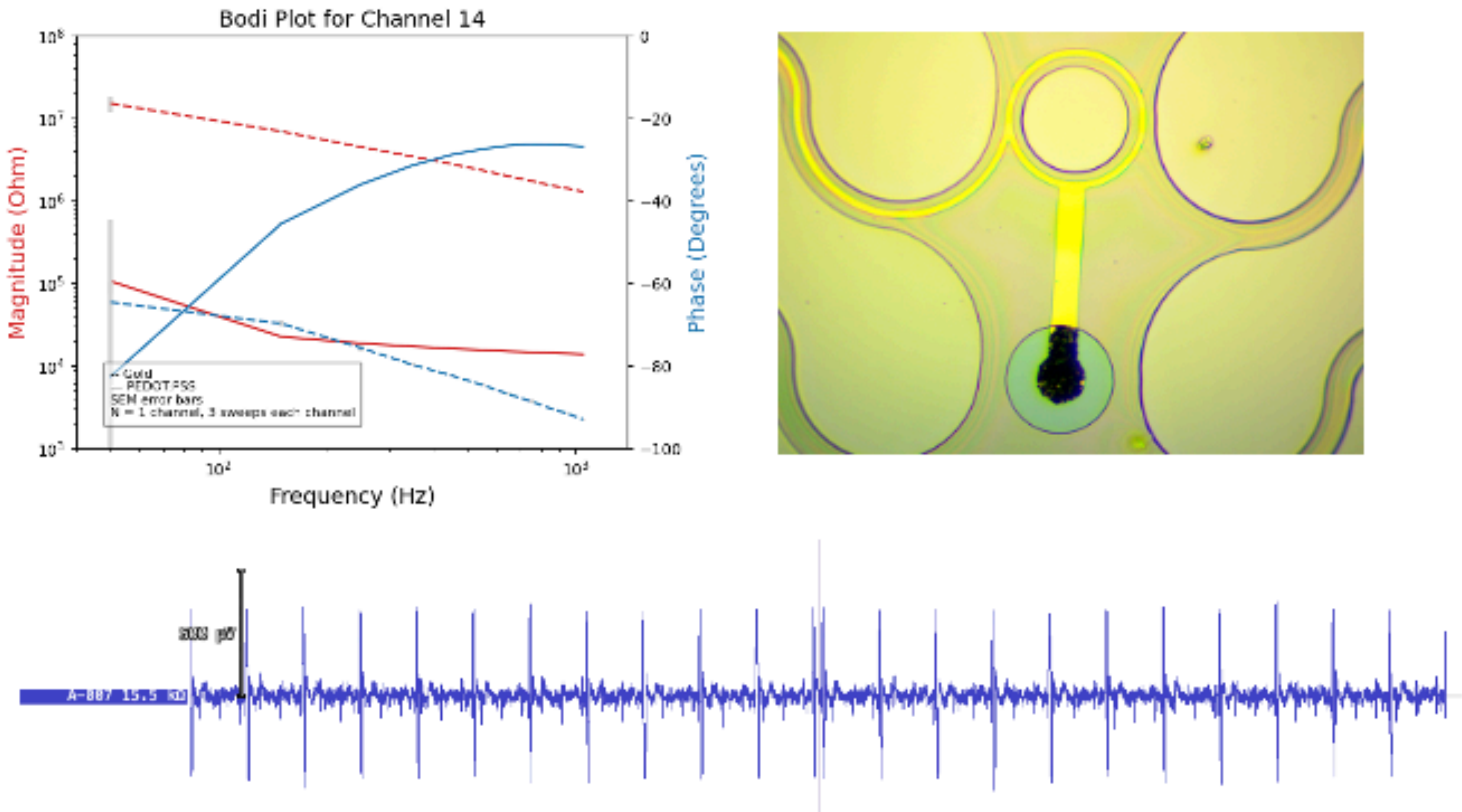

# 40 nA - 0 Min

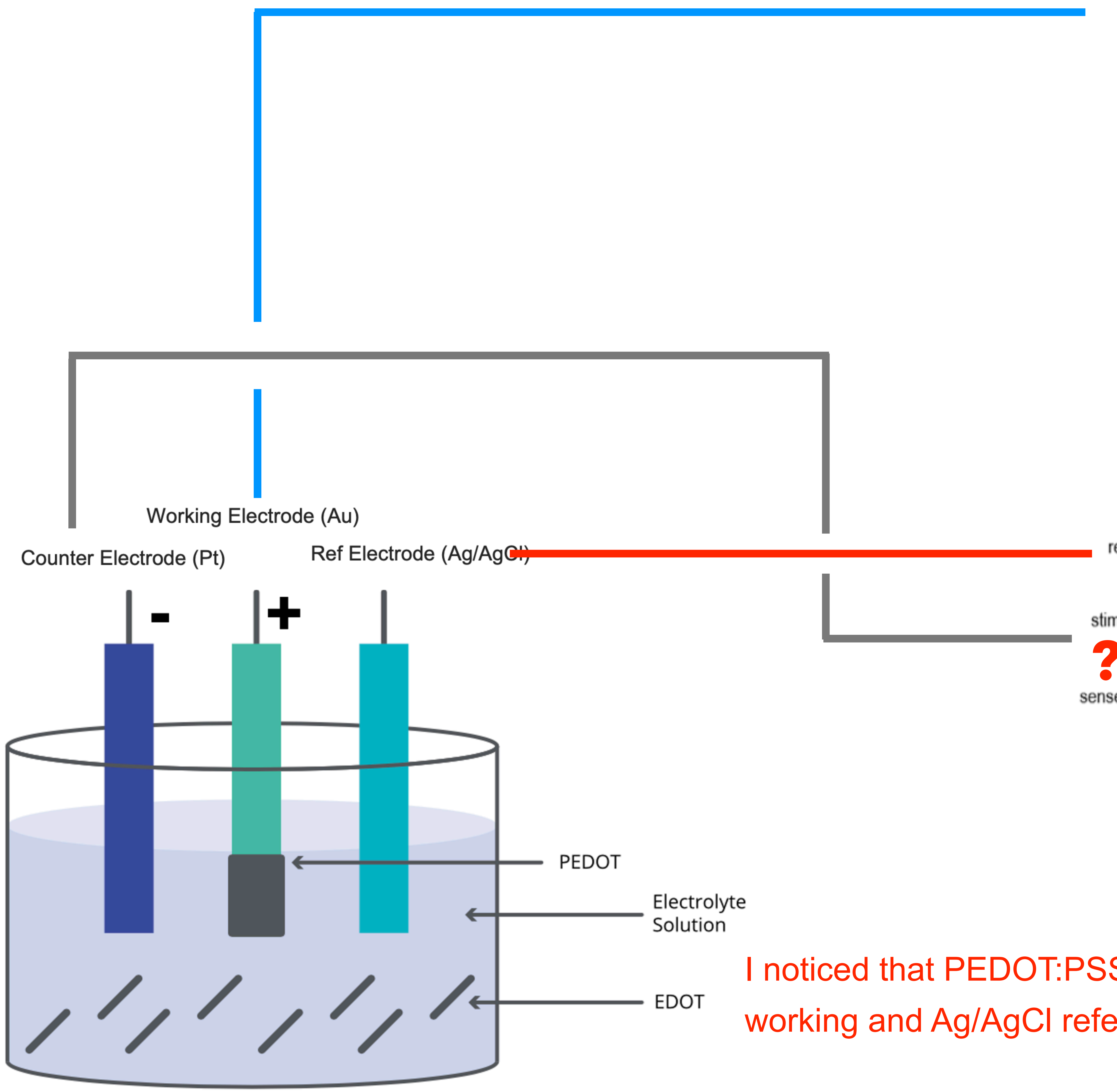


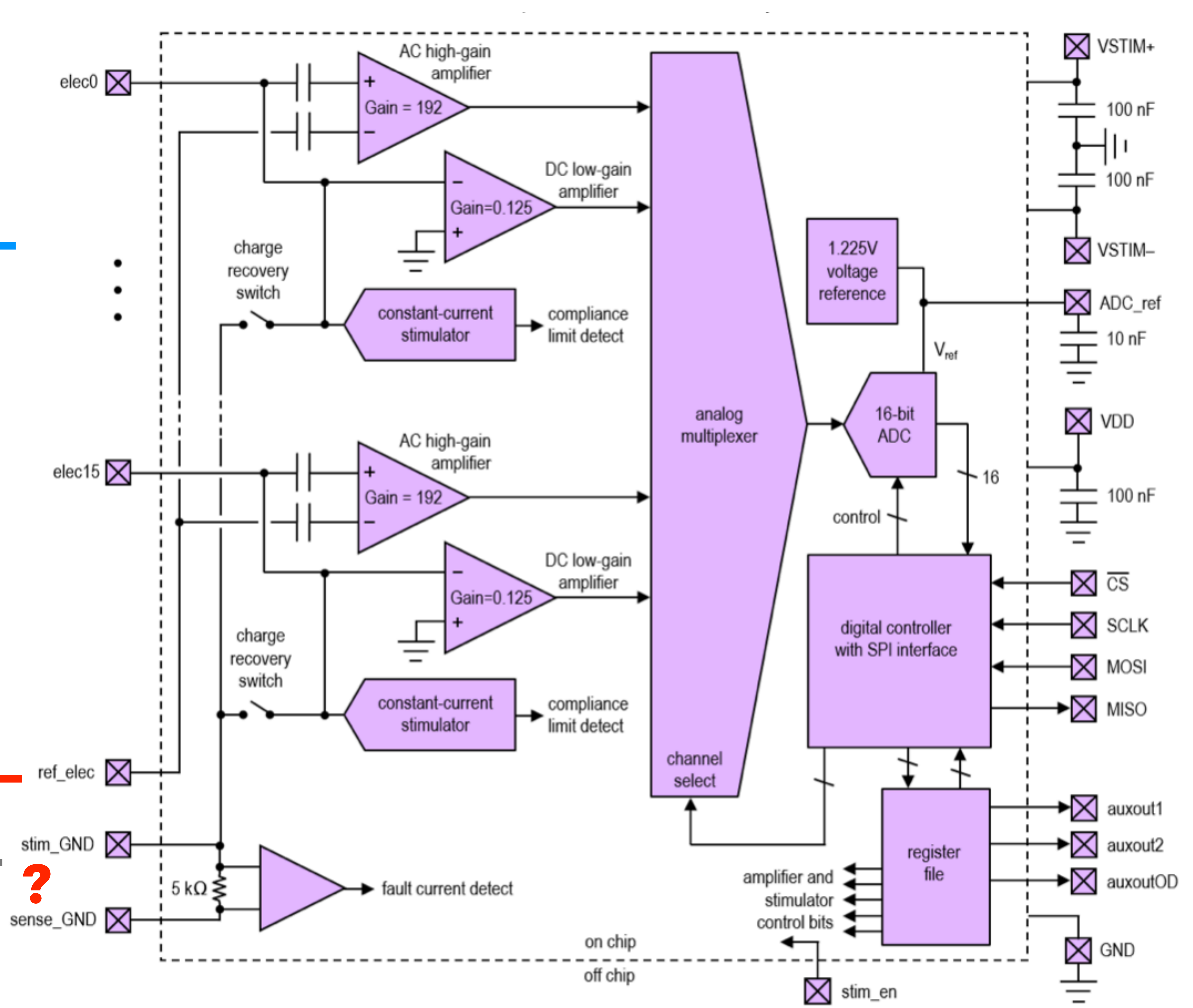

# 40 nA - 5 Min




# 40 nA - 10 Min






I noticed that PEDOT:PSS coated both the working and Ag/AgCl reference electrodes.

## Basic Components of Electroplating

1. **Electrolyte Solution**: A solution containing metal ions of the coating metal.
2. **Anode**: The electrode where oxidation occurs (positive electrode). It is typically made of the metal that will form the coating.
3. **Cathode**: The electrode where reduction occurs (negative electrode). This is the object to be plated.
4. **Power Supply**: Provides the necessary electric current for the electroplating process.

### *2.2. In Vivo Array Preparation*

Floating Microelectrode Arrays (FMAs, Microprobes for Life Science, Gaithersburg, MD, USA) were sterilized using an ethylene oxide gas sterilizer (AN 74i, Andersen Products, Inc., Haw River, NC, USA) after which they were transferred to a sterile environment. Initial quality-control impedance testing of all array sites was performed in a sterile PBS bath using a potentiostat (Autolab PGSTAT128N with FRA2 impedance spectroscopy module and Nova 1.8 control software, Metrohm USA, Riverview, FL, USA) with a platinum counter and Ag/AgCl reference (10 Hz–30 kHz, 10 mV RMS). If measured impedances differed substantially from manufacturer-reported values, the array was electrochemically cleaned (constant −2 V for 20 s). After cleaning, impedance was re-measured and cleaning repeated if necessary. Following testing, each array was immersed in a sterile polymerization solution prepared identically as that used in Kolarcik *et al.* [72], prepared as follows: prepared acid-functionalized MWCNTs and dexamethasone 21-phosphate disodium salt (Sigma-Aldrich) were dissolved into

## 1.23 volts

Water electrolysis requires a minimum potential difference of 1.23 volts, although at that voltage external heat is also required. Typically 1.5 volts is required. Electrolysis is rare in industrial applications since hydrogen can be produced less expensively from fossil fuels.

### 2.4.1. Cyclic Voltammetry

CV was performed using a PGSTAT128N potentiostat. Sequentially on each channel, CV was performed using 20 cycles from −0.9 V to 0.6 V (*vs.* Pt. counter electrode) at a 1 V/s scan rate, anode-first. Redox behavior of each site was qualitatively observed in terms of reduction and oxidation peak height and potential shift. Charge storage capacity and charge balance were computed by

### Components of the Three-Electrode Configuration

1. **Working Electrode (WE)**: This is the electrode on which the electroplating occurs. It serves as the cathode where the metal ions are reduced and deposited.

2. **Counter Electrode (CE)**: Also known as the auxiliary electrode, this serves as the anode. It completes the circuit by allowing the current to flow through the electrolyte solution. The material of the counter electrode is typically inert (such as platinum) or made of the metal being plated.

3. **Reference Electrode (RE)**: This electrode provides a stable and known reference potential. Common reference electrodes include the saturated calomel electrode (SCE) and the silver/silver chloride (Ag/AgCl) electrode. The reference electrode does not participate in the electrochemical reaction but allows for accurate control and measurement of the potential at the working electrode.

### Electroplating Process with Three-Electrode Configuration

1. **Preparation**: The working electrode (object to be plated) is cleaned and prepared. The electrolyte solution containing metal ions is prepared and the three electrodes are immersec in it.

2. **Connection to Potentiostat**:

   - The working electrode (WE) is connected to the working terminal of a potentiostat.
   - The counter electrode (CE) is connected to the counter terminal.
   - The reference electrode (RE) is connected to the reference terminal.

3. **Control of Potential**:

   - The potentiostat precisely controls the potential difference between the working electrode and the reference electrode.
   - By controlling the potential, the potentiostat regulates the reduction reaction at the working electrode, ensuring that metal ions are deposited at a controlled rate.

### Electron Movement and Electroplating in Three-Electrode Configuration

1. **At the Working Electrode (Cathode)**:
   - The potentiostat sets a potential relative to the reference electrode.
   - Metal cations in the electrolyte are reduced by gaining electrons supplied by the potentiostat.
   - The reduction reaction at the working electrode results in the deposition of metal atoms, forming the desired coating.
2. **At the Counter Electrode (Anode)**:
   - The counter electrode completes the circuit, allowing the current to flow.
   - An oxidation reaction occurs at the counter electrode, typically generating cations that go into the solution.
3. **Reference Electrode**:
   - The reference electrode maintains a stable potential, allowing the potentiostat to control the potential of the working electrode accurately.
   - It does not participate in the plating reaction but ensures the consistency and reproducibility of the electroplating process.