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Hardware Article

A low-cost and high-precision scanning electrochemical microscope built with open source tools



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ABSTRACT

A low-cost Scanning Electrochemical Microscope (SECM) was built with a 0.6 pA current measurement capability potentiostat and submicron resolution motorized stage, using open source software and hardware tools. The high performance potentiostat with a Python graphical user interface was built based on an open source project. Stepper motors, Arduino controller, a manual XY micromanipulator stage, 3D printed couplers and gears were used in building the motorized stage. An open source motor control software was used for moving the motorized stage with high precision. An inverted microscope was utilized for viewing a standard microelectrode while scanning. The setup was tested in the formation of a map of electrochemical signals from an array of pores on a parafilm membrane.

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Specifications table

Hardware name	Custom built SECM
Subject area	Biological Sciences (e.g. Microbiology and Biochemistry)
Hardware type	 Imaging tools
	 Measuring physical properties and in-lab sensors
Open Source License	CC BY-NC-ND 4.0
Cost of Hardware	<\$300
Source File Repository	https://osf.io/4us7t/

1. Hardware in context

There is an increasing interest in manufacturing custom laboratory research instruments with the simplified tools developed by the open source community. This approach has been spurred due to high cost and resulting lack of accessibility to high performance laboratory equipment in certain education and research institutions. The reduced cost and increasing

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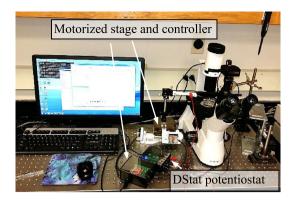


Fig 1. An image of the custom built SECM setup with custom built elements labeled.

availability of 3D printers and easy to program electronic boards are playing a key role in motivating researchers to build their own lab instruments [1]. Chagas and co-workers remarkably developed a whole open-source 3D printable platform for fluorescence microscopy, optogenetics and accurate temperature control which costs 100 Euros to build [2]. Various groups have been successful in the development of field compatible inexpensive potentiostats which work with smartphone applications [3,4]. Meloni and co-workers developed a 3D printed scanning electrochemical microscope (SECM) for a total cost of one hundred dollars [5]. A 5-µm resolution motorized stage was built from 3D printed parts that was employed in a screening microscopy [6]. Furthermore, this DIY approach not only provides an innovative solution to the lack of instrumentation accessibility issue, but also is a training opportunity for students in academic institutions to gain and develop design and troubleshooting skills during the process of building instruments [1].

Scanning Electrochemical Microscopy (SECM) is a powerful analytical tool for the identification of local electrochemical processes at various interfaces between gases, liquids and solids [7–10]. The commercially available SECMs are capable of carrying out nanometer resolution scans and sub picoampere current measurements. However, they cost several tens of thousand dollars. Here, we describe the procedure for building a high performance SECM with an inverted optical microscope, a custom-built motorized stage and a DStat potentiostat with 0.6 pA current measurement capability, where the cost of the custom-built parts was less than \$300. Fig. 1 shows a picture of the custom-built SECM with individual elements labeled. The setup has been successfully tested in standard electrochemical measurements, in the formation of an electrochemical signal image through scanning an array of pores on a membrane.

2. Hardware description

2.1. Potentiostat selection

A potentiostat is the core instrument of an SECM and its performance is a limiting factor for the type of measurements that can be conducted with the SECM. For example, battery research requires high voltage output but not low current measurements. The custom built SECM in this project will be used in the electrochemical detection of biomolecules, thus the low-level current detection capability was the determining factor in the selection of the potentiostat. Moreover, the overarching goal of the project was to custom build a low-cost SECM. However, the price range for commercially available standard potentiostats with low-current measurement capability is \$2000–\$20,000. There have been several attempts to significantly reduce the cost of the potentiostat with DIY approach, using open source programmable Arduino boards [11,12] and with other custom circuit board designs [3,4,13–15]. As shown in Table 1, the cost of Arduino based potentiostats [11,12] are in the \$30–40 range but they can only measure high microampere currents and they don't have the square wave voltage (SWV) measurement capability, which is required for the detection of low concentration analytes. Custom design circuit board based UWED and uMED potentiostats [3,4] have the advantage of having small-form factors as they are built for field applications, where cell phones and apps are used as interfaces. These potentiostats can also only measure high microampere currents. Other custom built potentiostats offer low microampere measurement capability and their cost range is \$80–100 [14,14]. In building the custom SECM in this project, the DStat potentiostat was chosen due to its superior low current (600 fA) measurement capability [15] and it still has a moderate cost of around \$120.

DStat potentiostat was built by following the detailed instructions provided by the developers in their publication, supplementary materials and the online project website [15,16]. The 3D printed DStat box was modified in our built and the new design is provided with this manuscript. The cost of the DStat potentiostat was around \$200 (a detailed bill of materials is provided as supplementary material), which is above the originally provided estimate (\$120) by the developers. As shown in Table 1, DStat potentiostat is capable of measuring sub picoampere signals, provides many different measurement modes, and its Python based user interface is very easy to use. Picoampere level currents were consistently measured with the custom built DStat potentiostat in various low-level signal experiments and some of them will be presented below.

Table 1Specifications of various custom-built potentiostats. *Reported cost by the developers [15].

Name Voltage Range and Resolution	Voltage Range and Resolution	Minimum Current and Resolution	Available Measurement Modes					Cost
		POT	CA	CV	DPV	SWV		
UWED	±1.5 V (67 μV)	±180 μA (6.4 nA)	/	~	~	✓	<i>1</i>	\$60
uMED	±2.0 V (50 μV)	±200μA (5 nA)	/	1	1	1	1	\$25
CheapStat	±1.0 V (NA)	±10μA	_	1	1	1	1	\$80
DStat	±1.5 V (46 μV)	600 fA	/	1	1	1	1	\$120*
Meloni et al.	±1.0 V (NA)	±200μA (NA)	/	1	1	_	_	\$30
JUAMI	±2.5 V (NA)	±10 mA (10 μA)	1	1	1	-	_	\$40
Dobbelaere et al.	±8.0 V (15.3 μV)	±2μA (1.2 pA)	1	1	1	-	_	\$100

2.2. Motorized stage

The reproducible scanning property of an SECM depends on its high precision motorized stage. Commercially available SECMs have nanoscale scan step capability through the use piezo motor stages, however, commercial piezo motor stages have starting prices of several thousand dollars and there is no established DIY approach literature on building piezo motors. Stepper motor-controlled stages provide micron scale resolution at a much lower cost, which is sufficient for the goals of this project. We have built a stepper motor-controlled stage with submicron step size and 2.5 cm range in both directions, using a manual XY stage, an Arduino board, two stepper motor driver shields, 3D printed parts and an open source user interface. An existing micrometer controlled manual XY stage was utilized in building the motorized stage, which can be purchased from various vendors. Others also reported on DIY approaches to building micromanipulators [17].

Fig. 2 shows a close-up image of the custom-built motorized XY stage. The choice of high-resolution stepper motors is key to achieving low step size, thus 0.9 deg/step stepper motors were utilized in building the motorized stage. As shown in the Fig. 2, the two-stepper motors were coupled to the manual XY stage via 3D printed parts and gears, where the difference in size and teeth numbers between gears enabled reduction of motor speed and hence the step size of the stage motion. The gear ratio was $N_{Large-5o}/N_{Small-13} = 3.84$, providing about 4 times reduction on the angular speed of each axis according to w_L - $N_L = w_S N_S$. The stepper motors were controlled with the Arduino board in conjunction with EasyDriver V44 shields, which resulted in further reduction of motor speeds by enabling adjustments to the supplied currents to the stepper motors. A custom Arduino shield was built using a perfboard to hold the two EasyDriver shields, the power jacks for stepper motors and the DC power input jack, where a 9.75 V DC adapter was utilized to power stepper drivers. A custom 3D printed box was used to house the Arduino board and the perfboard shield. A 5 V fan was also installed in the box to cool down the EasyDriver shields during operation. The open source GRBL software with a graphical user interface was utilized in sending commands

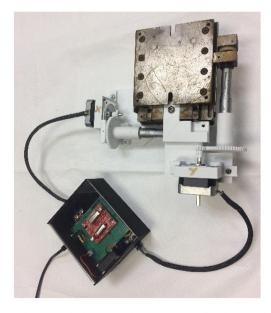


Fig 2. A close-up image of the motorized stage. The custom-built motorized stage parts including the manual XY stage, controller box, stepper motors, and 3D printed gears are shown.

to the stage for stepwise motorized scan of a preferred area [18]. This software is also capable of automatically moving the XY stage according to a user uploaded image pattern and an example is presented as a supplementary information movie (S2_video). GRBL software's website provides detailed instructions for establishing communication between the software and the Arduino board [18].

A detailed parts list, 3D printable part files, GRBL software operation instructions, and a summary cost break down for the motorized stage are presented in the below sections. A movie showing submicron step motion of the stage is provided as supplementary information (S1_video). We have also demonstrated controlling the Arduino board of the motorized stage through Python commands and in future upgrades, the motorized stage commands will be sent to the Arduino board motor controller through a single Python based interface, which will control both the motorized stage and the DStat potentiostat.

Once built, DStat potentiostat and the motorized stage can be used with any standard optical microscope to perform SECM experiments. An inverted microscope with enough clearance on top of its stage is preferred to accommodate the electrodes. The motorized stage is a modular bench top instrument and controls the motion of the working electrode of the DStat with arms extending to the top of the microscope stage as shown in Fig. 1. We have used a mixture of readily available standard optical components and a 3D printed electrode holder (supplied as a design file) for this purpose. However, standard optical adapters and metal bars can be easily replaced with 3D printed parts, which are available in online repositories. Our custom built SECM setup can only perform constant height scans as the motorized stage doesn't have Z direction (height) control. A future update will enable electrode height control with a separate motorized electrode holder, where the sample will be mounted on this existing motorized XY stage for obtaining SECM scans.

2.3. Highlights of the hardware

- A comparison between various custom built potentiostats were provided and it was determined that the DStat potentiostat is far more superior for low-level current detection experiments, hence a DStat potentiostat was built and utilized in the SECM setup.
- A motorized stage design method was provided, which is capable of moving in submicron steps. Other users can build a similar low-cost, custom motorized stage by using our design principles.
- Both DStat potentiostat and the motorized stage can be used individually for many different experiments.
- Information is also provided on how these custom-built instruments can be used in combination with a standard inverted microscope as a SECM setup, which can perform constant height scans.

3. Design files

A 3D printed motorized stage controller box is designed to house the Arduino board, the cooling fan, and the custom perf-board Arduino shield that holds the EasyDriver boards and a DC power jack. The box and the sliding front cover designs are universal and can be used by the DIY community without the need for modification. The sliding top cover for the motorized stage controller box was a 2.5 mm thick transparent acrylic plate in our project as shown in Fig. 3, which can be also replaced with a 3D printed version if desired. The other provided motorized stage 3D printable parts, including gears, bases, and brackets are designed to work with the manual XY stage that was readily available in our laboratory and as such they aren't universal. However, the DIY community can use them as guidelines to design their own motorized stage with their choice of (or a custom built) manual XY stage. The gears can be resized to be used with other types of manual XY stages.

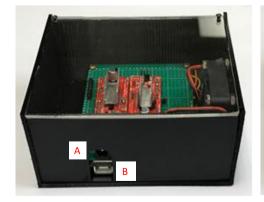




Fig 3. Images of the 3D printed box with the top transparent acrylic sliding cover. The labeled parts are A: DC power jack, B: USB jack, C: Fan ventilation, D: Stepper motor jack. The second stepper motor jack isn't shown in these images.

Design Files Summary

Design file name	ign file name File type Open source license		Location of the file
DStat Box	STL	CC BY-NC-ND 4.0	https://osf.io/kr4yp/
DStat front cover	STL	CC BY-NC-ND 4.0	https://osf.io/r3nsv/
MS-box	STL	CC BY-NC-ND 4.0	https://osf.io/h4xgr/
MS-box front cover	STL	CC BY-NC-ND 4.0	https://osf.io/ejrac/
MS-base1	STL	CC BY-NC-ND 4.0	https://osf.io/spv7t/
MS-base2	STL	CC BY-NC-ND 4.0	https://osf.io/ftygq/
MS-large gear	STL	CC BY-NC-ND 4.0	https://osf.io/sg7a9/
MS-small gear	STL	CC BY-NC-ND 4.0	https://osf.io/4m8zt/
MS-brackets	STL	CC BY-NC-ND 4.0	https://osf.io/3xy5s/
Electrode adapter	STL	CC BY-NC-ND 4.0	https://osf.io/vf2j9/

3.1. DStat box and front cover

The DStat 3D printed box was slightly modified compared to the original design [15]. In this new version, the DStat circuit board is mounted into the base of the box with screws. We have used a sliding transparent acrylic cover that is 2.5 mm thick, which makes on board LEDs visible during operation. This cover can be replaced with another 3D printed plate if desired. Our version of printed DStat box is shown in Fig. 1.

3.2. MS-box and front cover

The motorized stage (MS) controller box and the front cover are similar to the new version of the DStat box as shown in Figs. 1–3. We have also used a 2.5 mm thick sliding transparent acrylic cover for this box, which can be replaced with a 3D printed plate. The base of the box is designed to hold a standard Arduino UNO board. The box also houses a custom perfboard Arduino shield, which holds two EasyDriver boards and the DC power jack. Holes were drilled on one side wall of the box as depicted in Fig. 3 to provide airflow for the fan that is cooling the EasyDriver boards. The side walls of the box have also openings for the DC power jack, the Arduino board USB jack, and two female jacks for the stepper motor cables.

3.3. MS-base 1

This base has two functions: 1) to raise the manual stage from the surface for about 25 mm to provide clearance for the large gear on the coupler, 2) to enable coupling between one of the stepper motors and the microcontroller knob of the manual stage. The square shaped raised housing on this base holds the manual XY stage in fixed position. The stepper motor is also held in fixed position on this base with a separately printed bracket (MS-brackets.stl). This motor controls the X direction motion of the motor as shown in Fig. 2.

3.4. MS-base 2

This base is attached to the Y direction motion part of the manual stage with a bolt as shown in Fig. 2. The second stepper motor is attached to this base again using a separately printed bracket. The stepper motor and the gears move together with the top part of the manual stage as the X axis moves and provide Y direction motorized control at the same.

3.5. MS-large gear

Two of these gears were used for providing speed reduction. They slide over the microcontroller knobs of the manual stage as shown in Fig. 2.

3.6. MS-small gear

One of this gear was slid over each motor axle. This gear couples to the larger gear for speed reduction as shown in Fig. 2.

3.7. MS-brackets

This design contains two brackets, which are used to hold the stepper motors in fixed position on the bases.

3.8. Electrode adapter

The motion of the motorized stage is translated to the working electrode through extending arms, which are mainly standard optical components. This electrode adapter is used to attach a commercial working electrode with 6.3 mm diameter Teflon cylindrical body to a 10 mm diameter metal bar. A set screw and a small bolt/nut combination were used to establish tight connection to both the electrode and the metal bar.

4. Bill of materials

DStat potentiostat's reported cost was \$120 [15]. In our built, the total cost of the parts was about \$202 and a detailed bill of materials for all the parts are provided as supplementary information. The total cost of the DStat potentiostat doesn't include the cost of tools and supplies such as soldering iron and liquid solder. A hardware interface is needed for programming the microcontroller that costs about \$20 (included in the bill of materials). This interface is a tool that can be used in programming other microcontrollers, thus, it isn't included in \$202 total cost of building the DStat potentiostat. A bill of materials for building the motorized stage is presented below. The total cost of building the DStat potentiostat and the motorized stage for the SECM setup was less than \$300, excluding the cost of readily available manual stage, inverted microscope, some optical components and commercial electrodes.

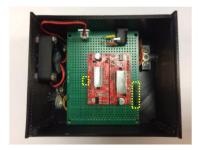
Designator	Component	Number	Cost per unit [USD]	Total cost [USD]	Source of materials	Material type
Stepper Motors	Vexta PK Series Hi Resolution (0.9 deg/step) NEMA 17 Stepper Motor (PK243M-03BA)	2	19	38	Amazon	Non-specific
EasyDriver Shields	EasyDriver – Stepper Motor Driver V4.4	2	15	30	Amazon	Non-specific
Cooling Fan	Daoki 12 V 404,010 mm Cooling Fan	1	1.5	1.5	Amazon	Non-specific
Perfboard	Double-Side Prototype PCB Universal Printed Circuit Board	1	1	1	Amazon	Non-specific
DC power adapter	9 V 1A Arduino Power Supply Adapter 110 V AC and jack	1	7.5	7.5	Amazon	Non-specific
PLA Filament	0.5 kg of PLA filament	1	8	8	Amazon	Polymer

5. Build instructions

Custom parts were printed using an ANET A8 3D printer. As described above, the DStat potentiostat was built according to the detailed instructions provided in its publication and project website [16]. Briefly, the PCB board was ordered from the same commercial vendor suggested in its publication and most of the 116 circuit components were installed with solder paste on a hotplate as they were surface mount style. Some of the circuit components were hand soldered on to the PCB board. After the completion of soldering, the board was tested for short circuits and functionality as described in its project website. The finished PCB board and the female banana plugs were installed into the 3D printed box with the modified design (DStat-box.stl and DStat front cover.stl). In the original DStat Box design, the PCB board slides into the box, whereas the board is secured in the bottom of the box with four screws in our design. The sliding transparent acrylic cover was secured on to the box with two screws as shown in Fig. 1. It should be noted that the contact with hotplate may cause burn hazard at elevated temperatures and solder fumes should be avoided with proper ventilation.

The motorized stage was designed around a readily available manual XY stage (Fig. 2) and below instructions can be used to replicate this work with any manual stage or by modifying 3D printable manual stage designs, which are available in online repositories. Manual stage is placed on the larger 3D printed base (MS-base1.stl), which provides height clearance for the large gear. The smaller base (MS-base2.stl) is attached to the Y-axis of the manual base with a bolt. The top base for holding the second stepper motor should be designed such that it is attached to the Y-axis without preventing the motion of the X-axis and this base should be able to move with the Y-axis together. The large gears (MS-large gear.stl) are attached to the microcontroller knobs of the manual XY stage and the small gears (MS-small gear.stl) are attached to the stepper motor axels. Set screws may be used if the 3D printed parts don't make tight contact with the knobs and axels. The stepper motors are aligned on the bases such that the gears make good contact with each other and they are fixed on the bases with screws and 3D printed brackets (MS-brackets.stl).

The Arduino Uno board was programmed to work with the open source GRBL interface by using the instructions and the code on GRBL project website [19]. The Arduino integrated development environment (IDE) interface was used for uploading



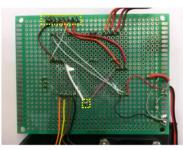




Fig 4. Images of the motorized controller perfboard shield circuit. (Left) Top view of the shield showing the EasyDriver boards and the DC power jack. The Arduino coupling pins are shown with yellow dotted line. The cooling fan and stepper motor jacks are also shown in this image. (Center) Bottom view of the shield depicts the soldered connections between DC power jack, cooling fan, EasyDriver board pins, stepper motor jacks and Arduino pins. (Right) Side view of the shield attached to the Arduino Uno board. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the code to the Arduino board. The GRBL software (version 1.0.9) was also downloaded from the project website and was installed on the controller computer. A custom Arduino shield was built for interfacing the two EasyDriver boards with the Arduino Uno board. Fig. 4 shows images of the perfboard shield holding the EasyDriver boards, the bottom view of soldered connecting wires and the shield with the Arduino board. The cooling fan is directly soldered to the DC power supply jack and turns on as soon as the controller box is powered. Two U-shaped custom metal plates were cut and placed on EasyDriver microprocessors with silicon grease to dissipate excessive heat generated during operation. EasyDriver board connections were made by soldering according to instructions on its project website [20]. The completed perfboard shield is placed on top of the Arduino board and the assembly is secured in the 3D printed box with screws (MS-box.stl and MS-box front cover.stl).

6. Operation instructions

- Connect the DStat potentiostat to the computer with the USB cable.
- Open command prompt on the computer.
- In the command prompt, type "activate dstat" and press enter key.
- Type "python -m dstat interface. main" and press enter key to open the DStat software.
- Once the software is opened, press "Connect" button.
- Choose the scan type and enter scan parameters on the left side panel of the software.
- Connect the working electrode, counter electrode and reference electrode to the corresponding channels on the DStat box. Use cables with an alligator clip on one side and banana type connector on the other end.
- Plug in stepper motor cables to corresponding jacks on the controller box.
- Power the motorized stage controller by plugging the DC power supply into jack A (Fig. 3).
- Plug in USB cable to Arduino board jack and the other end to the computer.
- Open GRBL software by running UniversalGcodeSender.exe.
- In the box labeled "Connection" on the top left corner of GRBL software (version 1.0.9), select correct Arduino communication port in the dropdown list, select 115,200 for baud rate and GRBL for Firmware. Click 'Open' button.
- If motor controller is successfully connected, the buttons in the "Machine Control" tab of the interface will become active and a message will be displayed in the "Command Table" tab.
- Enter step size in the "Machine Control" tab. The step size can be calibrated by using a microscope calibration slide and an inverted microscope as shown in the supplementary video (S1_video). For calibration, attach a sharp tip electrode to the motorized stage and place this electrode on the microscope calibration slide. Enter an arbitrary step size and click one of the buttons that moves the electrode along the horizontal direction as shown in the video (S1_video). Determine the distance covered with single click of a button for a given step size value. Use the measured value to adjust the step size. For our motorized stage, 0.09 step size corresponded to 1-µm motion of the electrode in both X and Y directions. Calibration is required only once and can be repeatedly used for other experiments.
- Set the desired step size for the motion of the electrode. Place the sample on the inverted microscope and place the working electrode on the desired start location for scanning. Counter and reference electrodes should be also immersed in the analyte solution of the sample as shown in the next section.
- Switch to DStat potentiostat interface and perform the desired voltammetry scan by pressing the "Execute" button at the bottom of the interface.
- To save the voltammetry scan data in spreadsheet text file format, click the following in order: "File" menu on the top, "save experiment data", choose the file where data should be saved, type the filename and press "Save." In order to save the data as an image in a pdf file, click the following in order: "File" menu on the top, "save plot", choose the file where data should be saved, type the filename and press "Save."

- Move to the next scan location using the GRBL software controls and repeat the two scan steps above in the DStat interface. Repeat until desired area scan is completed.
- To turn off the DStat software and the hardware, press "Disconnect" button at the bottom of the interface.
- In the command prompt, type "deactivate dstat" and press enter key.
- Unplug USB cable from the DStat hardware.
- To turn off the motorized stage, click the "close" button in the "Connection" box of the GRBL software. Disconnect the DC power adapter.
- The motorized stage can automatically follow a user chosen pattern, which can be uploaded under the "File Mode" tab. The GRBL software accepts pattern files with gcode extension and a custom pattern can be prepared with a variety of open source software such as Inkscape.
- GRBL software also allows the control of motorized stage through text commands, which can be typed under the "Console" tab.

7. Validation and characterization

7.1. SECM testing electrochemical signal mapping

The SECM was tested in forming an electrochemical signal image by scanning an array of 16 pores on a parafilm membrane as shown in Fig. 5a. As mentioned in Section 2.2 and as demonstrated in the supplementary video (S1_video), the motorized stage can move at submicron steps and it will be used in scanning a single live cell surface in future experiments. We are currently developing a nanoscale SECM electrode probe for this purpose, which isn't commercially available yet for performing such high-resolution scans. Here, we demonstrated the motorized stage functionality of the custom built SECM by scanning a 1.2 cm \times 1.2 cm area with 3 mm steps, where an ultramicroelectrode with 12.5 μ m tip diameter and 4 mm glass insulation diameter was used as the scanning working electrode.

A membrane was formed by stretching parafilm over a $2.5 \text{ cm} \times 2.5 \text{ cm} \times 5 \text{ mm}$ container filled with 5 mM K₃Fe(CN)₆ solution. The array of pores was formed by piercing the parafilm membrane with an electrochemically etched tungsten wire, which was prepared according to a previously established method in our lab [21]. Briefly, a 250micron diameter tungsten

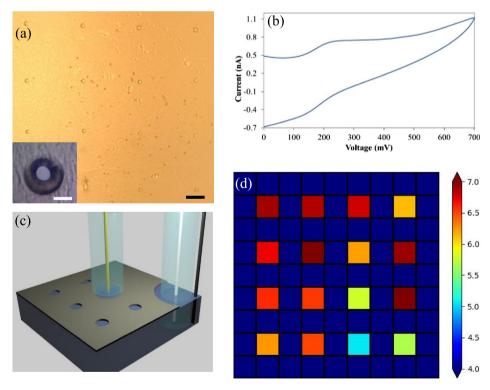


Fig 5. SECM setup testing in mapping electrochemical signals. (a) An optical image of the 4 by 4 array of pores on the parafilm membrane. Scale bar represents 1 mm. The average pore diameter was 80micron as shown in the optical image of a sample hole in the inset, where the scale bar depicts 100micron. (b) A sample K_3 Fe(CN)₆ cyclic voltammetry scan data from one of the pores in the array. (c) A computer rendered image of the experimental setup showing (not to scale) the working electrode over a pore and five other pores in the array. Reference and counter electrodes were immersed into the K_3 Fe(CN)₆ solution on the right corner of the container. (d) A map of the cathodic current value at 200 mV obtained from the scan of 81 different points covering 1.2 cm by 1.2 cm area around the array of 16 pores. The legend shows the nanolevel current signal color scale.

wire (A-M Systems) was immersed in 2 M NaOH solution in an oscillating fashion at 2 Hz frequency while applying 14 V DC potential between the tungsten wire and a counter electrode that resides in the NaOH solution until a tapered tip is formed.

In order to obtain the 4 by 4 array of pores, the motorized stage was utilized to move an etched tungsten wire with a pitch distance of 3 mm between pores, which was selected to match the diameter of the working microelectrode glass insulation tip diameter such that the working electrode doesn't record electrochemical signal in between consecutive pores. The working electrode was a 12.5 micron diameter commercial gold microelectrode (CH Instruments, CHI105) in 3 mm diameter glass insulation. Ag/AgCl reference electrode (CH Instruments, CHI111) and a 0.5 mm diameter platinum wire (Kurt Lesker) counter electrode were immersed into the K_3 Fe(CN) $_6$ solution through a wider opening in the parafilm membrane further away from the 80micron diameter pore array as depicted in Fig. 5c.

The motorized stage was used to move the working electrode with the GRBL software in scan steps of 1.5 mm to collect data from 81 different points in a $1.2 \text{ cm} \times 1.2 \text{ cm}$ area to cover all the pores in the array. In each step, the DStat potentiostat was used to record cyclic voltammograms of $K_3Fe(CN)_6$ solution (Fig. 5b), which is in contact with the working electrode only through solution leakage from a pore as shown in the diagram in Fig. 5c. The magnitude of the cathodic current at 200 mV was extracted from 81 data points, which was used in preparing the spatial electrochemical signal map around the pores as shown in Fig. 5d. The cyclic voltammetry measurements yielded noise level signals in between pores as the trapped K_3Fe (CN) $_6$ solution under the working electrode didn't contact the solution below the parafilm membrane at these locations. A variation in the signal amplitude was observed from pore to pore most likely due to non-uniformity of pore diameters. This result demonstrated the capability of the motorized stage in locating the pores precisely, which is necessary for proper functioning of the SECM.

7.2. Conclusions

This work demonstrates the feasibility of building essential parts of a high performance SECM setup for under \$300 with DIY approach. The custom built SECM setup was tested by forming an electrochemical signal map from an array of pores in a membrane. This SECM setup is not only a cost-efficient instrument but also its development is a hands-on training project for students on electronics, mechanics and electrochemistry. The modular nature of the setup also enables the utilization of individual components such as the potentiostat and the motorized stage for other experiments as needed. The current SECM is designed for constant height scans and the Z-height control with automated electrode approach function will be added in future updates. This custom-built SECM setup will be utilized in obtaining high-resolution electrochemical signal maps from individual cells with the use nanoscale electrodes in the future experiments.

Summary of capabilities and limitation of the SECM setup:

- 500 fA current detection capability.
- Cyclic, square wave voltammetry, chronoamperometry scans.
- Submicron (500 nm) scan step size.
- Motorized stage is capable of following a user defined pattern.
- Currently, the SECM setup can only perform constant height scans as there is no Z height control, which will be included in a future update.

Human and animal rights

This work doesn't involve the use of human and animal subjects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/i.ohx.2019.e00082.

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