Electrochemical analysis of laser-inscribed graphene electrodes using cyclic voltammetry (ferri/ferrocyanide redox couple)

Yifan Tang[1], Lisseth Casso Hartmann[2,3,4], David Bahamon Pinzon[2,3,4], Geisianny AM Moreira[2,3], Diana Vanegas[2,3,4], Eric S McLamore[2,3,4,5]

[1]Plant and Environmental Sciences, Clemson University;
[2]Department of Environmental Engineering and Earth Sciences, Clemson University;
[3]Global Alliance for Rapid Diagnostics, Michigan State University;
[4]Interdisciplinary Group for Biotechnology Innovation and Ecosocial Change-BioNovo, Universidad del Valle;
[5]Agricultural Sciences Department, Clemson University

Eric S McLamore: corresponding author;

SNAPS research group

Eric S McLamore
Clemson University, North Carolina State University

FEB 12, 2023

DOI:
dx.doi.org/10.17504/protocols.io.4r3l27q7jg1y/v1

Protocol Citation: Yifan Tang, Lisseth Casso Hartmann, David Bahamon Pinzon, Geisianny AM Moreira, Diana Vanegas, Eric S McLamore 2023. Electrochemical analysis of laser-inscribed graphene electrodes using cyclic voltammetry (ferri/ferrocyanide redox couple). **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l27q7jg1y/v1

Protocol status: Working
We use this protocol and it's working

Created: Nov 28, 2022

Last Modified: Feb 12, 2023

PROTOCOL integer ID: 73291

DISCLAIMER

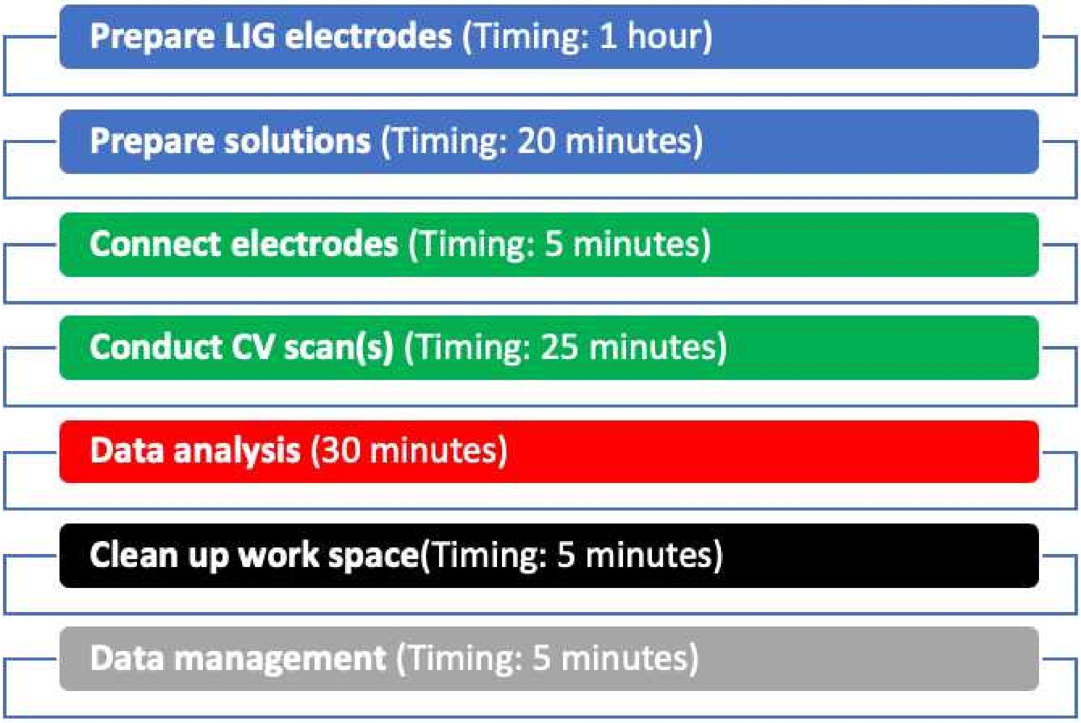
The authors acknowledge the National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health under Award Number U01AA029328, the National Institute of Food and Agriculture (2018-67016-27578) and the National Science Foundation (CBET no. 1805512). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, National Institute of Food and Agriculture, or National Science Foundation.

ABSTRACT

This protocol describes use of the cyclic voltammetry (CV) method for electrochemical analysis of laser-inscribed graphene (LIG) electrodes. The protocol requires approximately 1 hour (excluding electrode fabrication).

GUIDELINES

The steps for the process are summarized in **Figure 1**. The protocol is based on numerous published manuscripts that use similar methods with the ferri/ferrocyanide redox couple (REF 1-4), but is not a comprehensive guide. Where appropriate, alternative redox couples or electrolytes are noted.



Process flow for conducting electrochemical analysis of laser inscribed graphene electrodes using cyclic voltammetry. The protocol is organized by sections: preparation (blue), electrochemical analysis (green), data analysis (red), cleanup (black), and data management (grey).

MATERIALS

●LIG electrodes (see protocol for LIG fabrication above)
●Potassium ferrocyanide ($K_4[Fe(CN)]_6$) (link to SDS)
●Potassium ferricyanide ($K_3[Fe(CN)]_6$ (link to SDS)
●Potassium chloride (KCl) (link to SDS)
●Auxiliary electrode. This protocol uses a 7.5 cm long platinum wire or a 3-electrode LIG chip as noted.
oCatalog number for single platinum wire electrode: MW-1032, BASi, West Lafayette, IN, USA (link here)
●Reference electrode. This protocol uses a Ag/AgCl with 3M KCl internal solution or a 3-electrode LIG chip as noted.
oCatalog number for single reference electrode: MF-2056, BASi, West Lafayette, IN, USA) (link here)
●Optional: QuadHands magnetic workbench (KOTTO, Tokyo, Japan) (link here)
●Optional: If 3-electrode chip is used, additional materials include:
oUSB type A connector (link here)
oChemical resistant PVC backing (12" x 12" x 1/16"; McMaster-Carr part no. 8747K111) (link here)
oDouble sided clear tape (link here)

HARDWARE

●Magnetic stir plate (link here)
●MultiPalmSens4 potentiostat (PalmSens, Houten, Netherlands)
●Optional: ABE-Stat (Diagenetix, Honolulu, USA) and a Tablet or (Android) cellphone

SOFTWARE

●MultiTrace4 software for PalmSens4
●ABE-Stat app if using ABE-Stat potentiostat

SAFETY WARNINGS

SAFETY
●Lab coat, gloves, and closed-toed shoes are mandatory
●Nitrile gloves (powder-free type as applicable)
●Wrist grounding strap (optional) (link)

Chemical safety hazard
●All chemicals should be handled with gloves and a lab coat.
●When using scale to weigh chemicals, ensure the residues are cleaned and discarded in the solid chemical waste container as described by the solid waste disposal procedure and in reference to the SDS for all

chemicals.
●After using electrolyte or other solution, for future use, store the solution in a capped glass bottle in 4^{o} C fridge or discard them in a labeled waste solution container.

Eye protection
●Goggles or eye protection is required when handling solutions outside of a chemical hood.

Skin
●If any solutions are spilled and encounter skin, immediately rinse under water and wash with soap for at least 5 min and follow procedures in SDS (links provided in material section).

Disposal
●CV solution should be discarded in the appropriately labeled waste container in the satellite accumulation area and not disposed of in the sink or the trashcan.
●Used LIG electrodes should be discarded in a labeled waste container sorted by appropriate hazard class (e.g., biohazard solid waste if applied for detecting pathogens, heavy metal solid waste, etc.).

ACCESIBILITY

The following guidance is summarized from Perry and Baum [5] for ADA laboratory (building) compliance,where relevant to this protocol.

General building codes for laboratory

- Minimum 2 Exits for labs ≥500 ft^2 net area.
- Minimum 2 Exits for labs using chemical fume hoods or glove box
- Minimum 2 Exits for labs using flammable and combustible: liquids, gases, cryogenics, dusts and solids.
- Minimum 2 Exits for labs using oxidizers, unstable reactives, water reactives, organic peroxides, highly toxics, corrosives.

Wheelchair clearance must be provided as follows:

- Egress for wheelchair 360° Turn is 1.5 m (5 ft) clearance.
- Both sides of Exit and Entry doors to
- Emergency Eyewash & Safety Shower
- In front of wall benches, sinks, equipment
- In front of chemical fume hoods
- At chalk/marker board
- Between benches
- Aisles that lead to Primary Exits, back to front
- Aisles that allow passage side to side in lab

Standard accommodations for use of chemical hood or other exhaust air systems

- knee space obstructions
- adjustable work surface height
- accessible receptacles and alarm control

Common equipment

- Where visual inspection is utilized, alternative technologies should be listed as optional (colorimeters, spectro-radiometers, etc.)

BEFORE START INSTRUCTIONS

Always wear proper PPE during experiment (gloves, eyewear, lab coat, proper clothing)

Preparation

1h 20m

1 **Prepare LIG electrodes**

1h

- Use protocol for LIG fabrication to prepare a batch of electrodes (link here).
- For quality control process to prepare large batches of LIG electrodes, contact the corresponding author (emclamo@clemson.edu).

Note

Wear eyewear protection, gloves, and a lab coat during all experiments.

Expected results from step 1:

- **Fig 1** shows photographs of the instrument and the expected results from a batch fabrication of six replicate electrodes based on the LIG fabrication protocol here

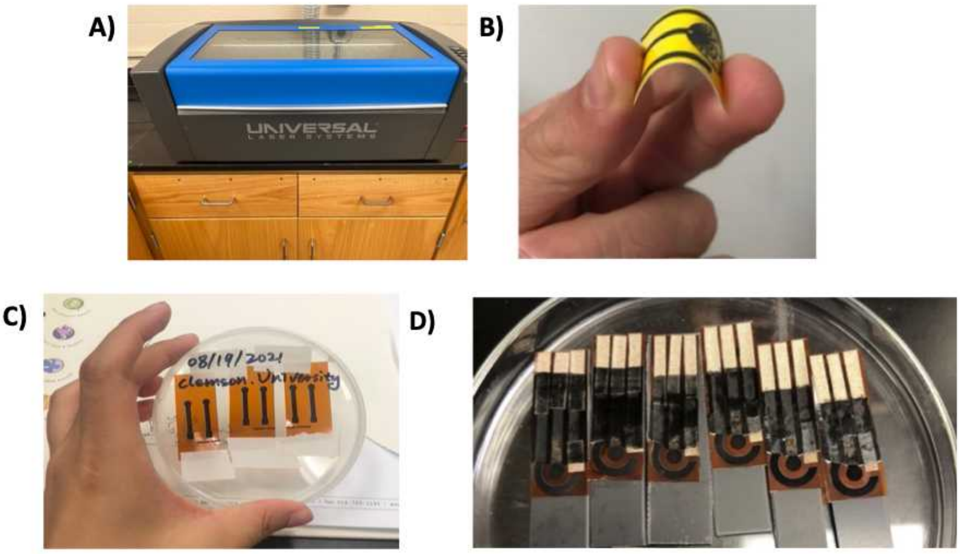


Figure 1. LIG fabrication protocol used to produce a batch of electrodes. **A)** Universal laser system used in the fabrication of LIG electrodes. **B)** Flexible LIG electrode. **C)** Representative batch of six replicate LIG electrodes. **D)** Representative batch of six replicate LIG biochips.

Note

Store electrodes in a sealed Petri dish or small container until ready for analysis. Be careful not to place heavy or sharp objects on top of the LIG electrodes

2 Prepare solution

20m

- Gather a sealed glass storage bottle or Falcon tube with at least 50 mL of capacity and label as 2.5 mM ferri/ferrocyanide + 100mM KCl. The masses below are for preparation of a 50mL solution.
- In a separate weigh boat/paper, prepare the following:

  1-Weigh 52.8 mg potassium ferricyanide (2.5 mM $K_4[Fe(CN)]_6$) on a scale.

  2-Weigh 41.2 mg potassium ferrocyanide (2.5 mM $K_3[Fe(CN)]_6$) on a scale.

  3-Weigh 373 mg potassium chloride (KCl) on a scale.
- Add the three powders to the labeled glass bottle
- Fill glass storage bottle to 50mL with DI water (or nano-pure water)containing 10mL

Note

A stock solution of 1M KCl may be prepared in advance and stored between 4 and 25°C until used. Do NOT prepare stock solutions of redox reagents in advance, as they will undergo reaction and not be viable for the experiment.

- Place clean magnetic stir bar in bottle and place on a stir plate
- Stir for 5 minutes at 1,500 rpm
- After mixing, inspect the bottle to ensure dissolution
- Transfer 20 mL of the solution to the electrochemical cell (20 mL cell shown in **Fig 2**)
- Prior to analysis, inspect the solution to ensure the color is appropriate. The solution should be yellow in color (not clear). If preferred, a cell phone app may be used to detect the color of the electrolyte. For example, Color Name AR is a helpful tool for color analysis that is available for both iPhone and Android systems (https://apps.apple.com/us/app/color-name-ar/id906955675).

Note

Although this protocol uses KCl, other electrolytes may be used. In general, the electrolyte concentration should generally be greater than 10 μM to avoid migration effects. The electrolyte concentration has an optimum window that should be studied carefully [8].

Electrochemical testing

30m

3 Connect electrodes

5m

Note

- Two different types of LIG electrode connections are shown in this protocol: i) a single LIG electrode versus commercial auxiliary/reference electrode, and ii) three electrode LIG chip. See protocol by Casso-Hartmann et al (2023) for details.
- Two different instruments are shown in this protocol: i) a benchtop potentiostat, and ii) a portable potentiostat (known as ABE-STAT)

Assemble electrochemical cell (Benchtop Palmsens4 system):

Single electrode system

- Connect the single LIG electrode with the potentiostat via the bonding pads using alligator clips
- Ensure the electrodes are not touching, and the distance between the auxiliary and working electrodes is consistent.
- **Fig 2A** shows the correct assembly of the electrochemical cell for a single LIG "dip-style" electrode, where a Ag/AgCl reference electrode and Pt wire are used as reference and counter electrode, respectively

Three electrode (chip) system
- Connect the USB adapter to the chip directly, and position the LIG chip in the electrochemical cell using the QuadHands magnetic workbench (**Fig 2B**).
- For connecting the chip system, a female USB type A connector is used as described in the LIG fabrication protocol (see **Fig 2C**).

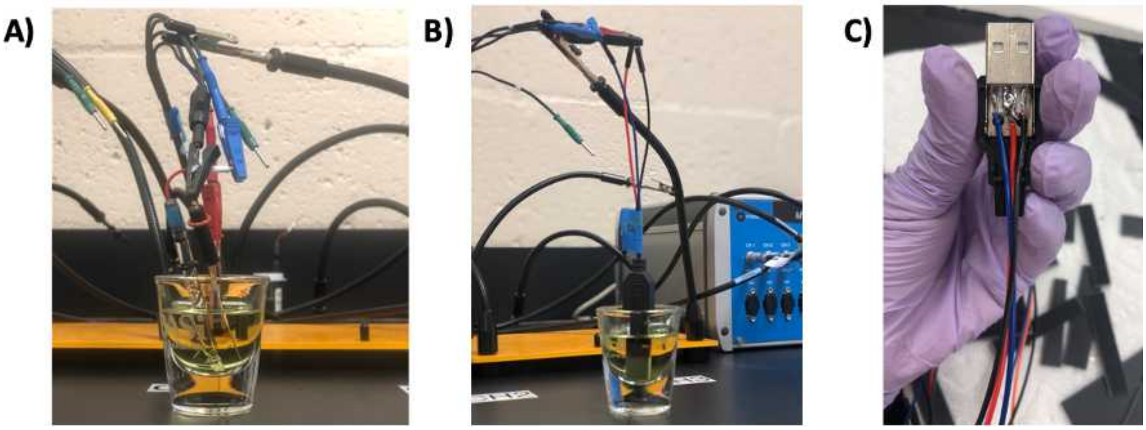


Figure 2. Connecting LIG electrodes (single LIG working electrode and LIG 3 electrode chip) to benchtop potentiostat (PalmSens4). **A)** Correct assembly of the electrochemical cell for a single LIG electrode, with external reference and auxiliary electrodes. **B)** Correct assembly of the electrochemical cell for a three-electrode system LIG chip. **C)** Assemble USB connector by soldering wires into connection pins as described by Casso-Hartmann et al (2023)

Note

If using a single electrode, the distance can be an important factor in performance as discussed by Bentley et al. Thus, consistency is critical

Assemble electrochemical cell (ABE STAT portable system):
- The ABE-STAT contains indicators for connections of working (W), counter (C) and reference (R) electrodes (**Fig 3A**).

- Connect the electrodes accordingly, as described previously. **Fig 3B** shows an example of an experimental setup using a three-electrode chip.
- The electrodes are positioned in the electrochemical cell using the QuadHands magnetic workbench.
- A single electrode, or chip system may be developed for ABE-STAT, as shown in **Fig 2.**

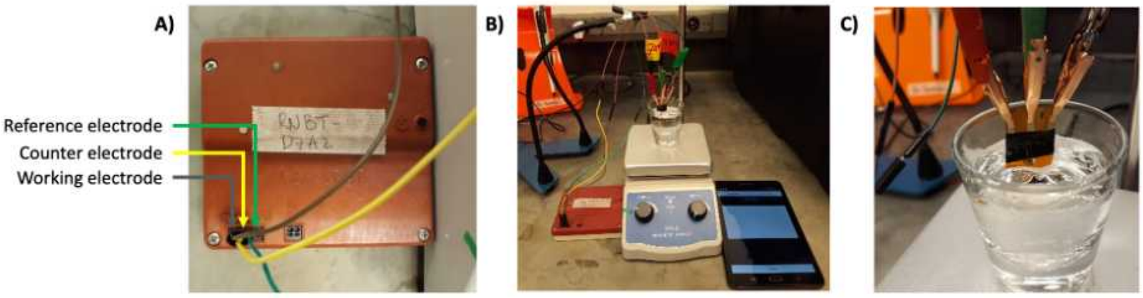


Figure 3. Set up of the ABE-Stat. **A)** Connections to the ABE-Stat. **B)** Example of experimental set-up with portable potentiostat and tablet connected through Bluetooth. **C)** Exploded view of electrodes immersed in electrochemical cell

4 Conduct scan (PalmSens4 benchtop system)

25m

Note

To ensure that electrodes are positioned correctly (particularly dip-style single electrodes), a QuadHands workbench or similar setup is recommended.

- Turn on Palmsens hardware and the computer.
- Open the PalmSens software (MultiTrace4 software).
- Users may operate 3 channels simultaneously or individually (**Fig 4C**)Select Cyclic Voltammetry as the Technique (**Fig 4D**). After selecting the mode, set up all parameters. For example, see settings below (see MultiTrace manual for details; link here)

Note

Cyclic Voltammetry settings

- t equilibration: 3s
- E begin: -0.8 V
- E vertex1: -0.8V
- E vertex2: 0.8 V
- E step: 0.01V
- Scan rate: 0.2 V/s
- Number of scans: 10

- Parameters may be modified based on experiment needs. For example, when conducting experiments to determine the electroactive surface area (ESA), at least four scan rates are suggested. See references for details

Expected results from PalmSens4 benchtop potentiostat

- **Fig 4** shows the channels connections to the PalmSens, mode selection panel, and operation panel.
- When the experiment finishes, click export to excel icon to export data.
- When exporting data, make sure all channels' data are exported.

Data analysis

5 **Analyze CV data**

- CV data are commonly analyzed to produce the following outcomes. References are provided for details of calculations.
- Electroactive surface area [8]
- Charge, if a pseudo-capacitor or capacitor [9]

Expected results

- **Fig 8** shows a representative cyclic voltammogram for a single LIG electrode.

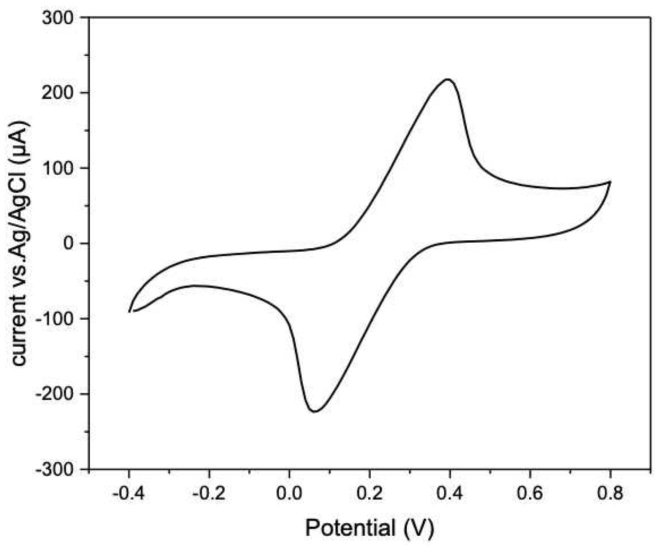


Figure 6. Representative cyclic voltammogram for a single LIG electrode.

Note

- Ferri/Ferrocyanide is a negatively charged redox probe. Other redox probe solutions may be used, such as ruthenium ammonium chloride (positively charged redox probe), hydroquinone (neutral probe), or other alternatives as described in the literature

Clean up

6 **Clean workspace (Timing: 5 min)**

- Ensure chemicals are stored or discarded correctly, and turn off Palmsens and the computer.
- If using external reference and auxiliary electrodes, clean them up with DI water and store them in the appropriate place. Reference electrodes should be stored with a 3M KCl solution.
- Wipe the working desk with 75 % alcohol.
- Store electrodes when not in use.

Data Management

7

File naming: For files from CV analysis, the following naming system was utilized: DATA_CV1.1

File storage: Store all methods in the Desktop folder with the operator's name clearly identified in the folder name.

Backup files: At least once per year, ensure that the folder is backed up on the lab's external hard drive.