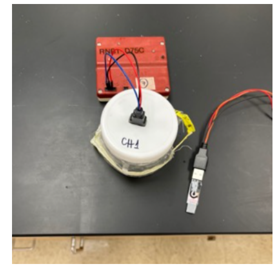
Jun 05, 2024 Version 2

Fabrication of laser inscribed graphene (LIG) 3-electrode plug-and-play chip V.2



DOI
dx.doi.org/10.17504/protocols.io.dm6gpze1dlzp/v2

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

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

Eric S McLamore
Clemson University, North Carolina State University

DOI: **dx.doi.org/10.17504/protocols.io.dm6gpze1dlzp/v2**

Protocol Citation: Lisseth Casso-Hartmann, Geisianny AM Moreira, Yifan Tang, Diana Vanegas, Eric S McLamore 2024. Fabrication of laser inscribed graphene (LIG) 3-electrode plug-and-play chip. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.dm6gpze1dlzp/v2**Version created by **Eric S McLamore**

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

Created: March 24, 2024

Last Modified: June 05, 2024

Protocol Integer ID: 101268

Keywords: Laser inscribed graphene, LIG, electrode, chip, plug-and-play

Funders Acknowledgement:
National Institutes of Health
Grant ID: U01AA029328
Clemson University
Grant ID: 2021001151
National Science Foundation
Grant ID: CBET: 2019435

Disclaimer

Research reported in this publication was supported by the National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health under Award Number U01AA029328 (DV). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Additional support was provided by the COVID Research Seed Grant Program, Clemson University (Award #2021001151), and the Startup Funding from the Department of Environmental Engineering and Earth Sciences of Clemson University (DV). NIH P20GM121342 supported sequencing efforts (DD). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Laser inscribed graphene (LIG) is a versatile material that is commonly used to prepare electrochemical sensors (Moreira et al 2023). This protocol describes the fabrication of a 3-electrode plug-and-play chip system based on LIG. The design is compatible with a female USB-A connector which can be modified for connection to any potentiostat using the protocol here. The complete process requires approximately 60 min to complete, including fabrication of USB-A connector (**Fig 1**).

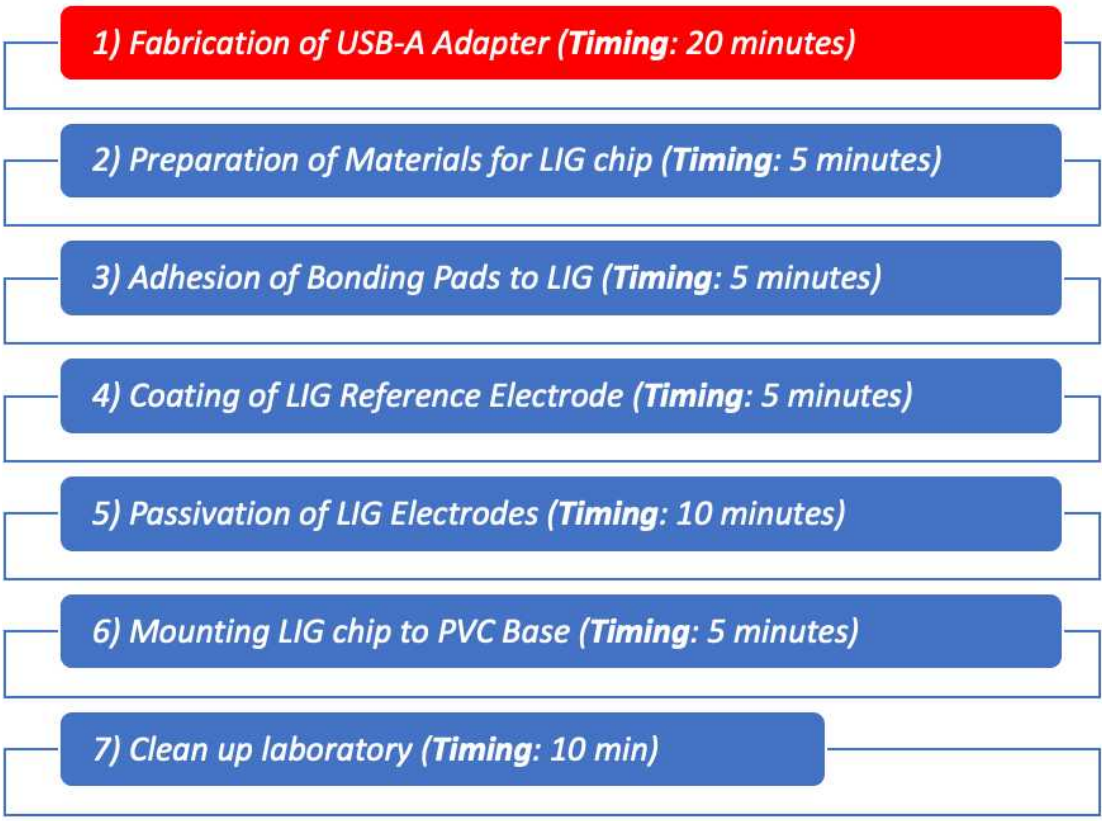


Figure 1. Process flow for fabrication of laser inscribed graphene (LIG) chip compatible with USB-A. The process shown here is for a batch of 18 LIG chips, and requires approximately 60 min to complete. The USB-A adapter may be reused, and thus is shown as a separate section. The protocol depends on referenced protocols for fabrication of LIG (not shown here).

Image Attribution

Plug and play system is compatible with any potentiostat and can be directly integrated with sample container(s)

Materials

Hardware

- Hardware required for fabrication of LIG using Universal Laser, see protocols here (**link**)
- Soldering station (**link**)

Software

none

Chemicals, Reagents and Other Materials

- Type HN Amber plain-back polyimide film (0.005" thick") (**link**)
- 70% Isopropyl alcohol (**link**)
- Low-Lint Cloth Wipes (**link**)
  Masking Tape (**link**)
- Conductive polyester metal tape (0.13 mm thick) (**link**)
- Double-sided film tape (**link**)
- Chemical-resistant PVC Sheets of 1/16" thick, 12X12 sheet **(Link)**
- Certified eyewear **(Link)**
- USB-A Female Port Socket with Plastic Cover (**link**)
- 28AWG jumper wires, PVC insulation (**link**)
- 395 nm UV flashlight (**link**)
- Scissors (local store)
- Steel tweezers (local store)
- Fast Drying poly gel acrylic nail polish (UV curing) (local store)
- Timer (local store)

Safety warnings

Eye protection

- Laboratory eye protection is required when cutting PVC, as small shards may cause eye damage during the preparation of electrode base material process.

Skin

- Avoid contact of acrylic nail polish with skin. Proper use of PPE should avoid any problems (**link to health hazard information**)\

Fumes/aerosols

- All use of the following chemicals must be conducted in a chemical hood or in a well-ventilated space
- Acrylic nail polish (or other types of nail polish (**link to health hazard information**)
- Isopropyl alcohol (**link to SDS**)
- All soldering should be conducted in a chemical hood to avoid iron based solder fumes

Heat and Flammable materials

- Avoid open flame or source of ignition when working with acrylic nail polish with skin (**link to health hazard information**)

Disposal

- Any excess polyamide,
  metal tape, or PVC sheet should be discarded in a labeled container on the
  counter.

Ethics statement

N/A

Before start

Ensure ample table space is available, and located in a well ventilated area.

Safety information

- Wear eyewear protection at all times
- Wear gloves and lab coat at all times

1 *Step 1) Fabrication of USB-A Adapter* 20m

- Heat the soldering iron, and prepare a wet sponge and solder wick.
- See the following for an introduction to soldering techniques (**link**)
- Solder one 28AWG jumper wire to each of the two outer contacts in the USB-A adapter.
- Create an electrical connection (i.e., “jump”) the two inner contacts in the USB-A adapter.
- Solder one 28AWG jumper wire to the “jumped” inner contact(s) in the USB-A adapter
- Place the plastic cover on the USB-A Female adapter
- Turn off soldering iron, and clean station when finished

2 *Step 2) Preparation of Materials for LIG chip* 5m

- LIG should be prepared using the protocol developed for a 3-electrode sensor (**link**)
- Using scissors, cut conductive polyester metal tape into 0.3 cm by 3.5 cm strips (one strip per LIG electrode)
- **Fig. 1** shows an example of a batch of 18 LIG electrodes with 18 metal tape strips
- For each LIG chip, cut a 1cm by 5 cm strip of PVC to act as the chip base
- Ample table space should be cleared to prepare the LIG chips

Note

Critical Step:
Ensure that the PVC base is the correct width by inserting into the USB-A female adapter. The fit should not be too loose, but should be snug when inserted.

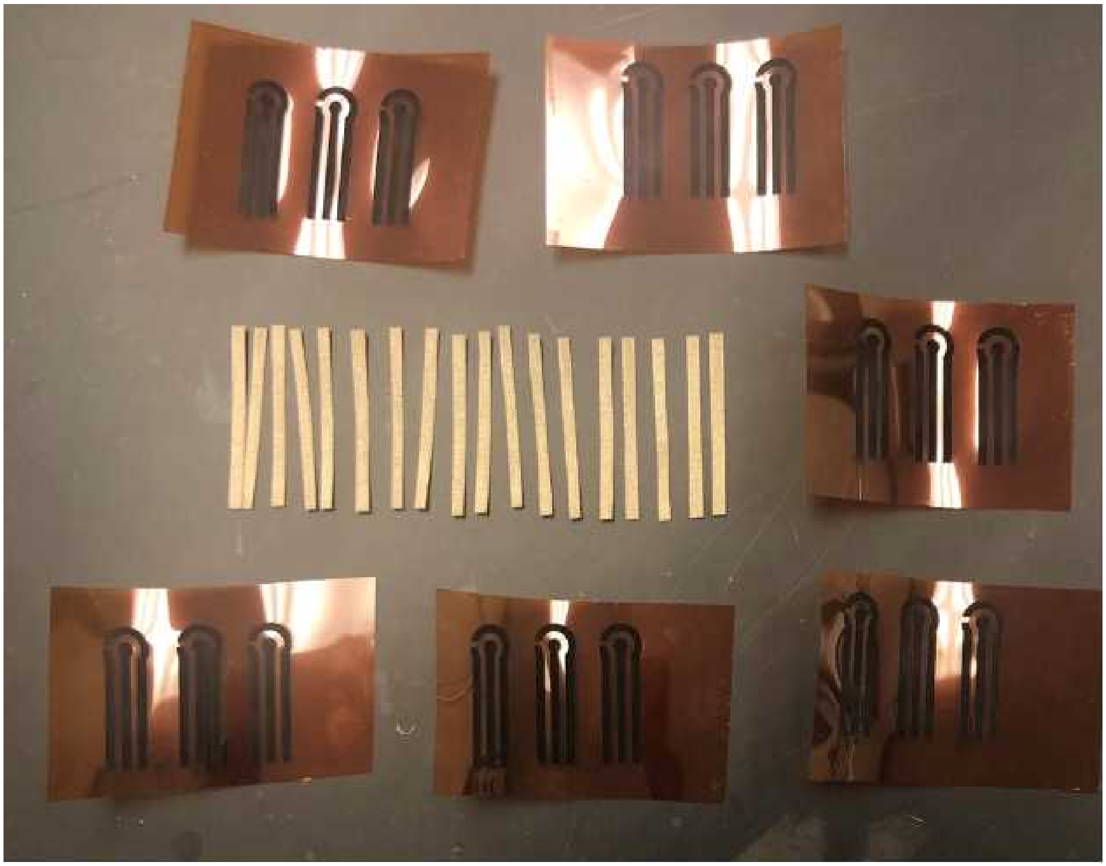


Figure 1. Photo of materials prepared for a batch of 18 LIG electrodes with 18 polyester metal tape strips (PVC base strips not shown in photo)

3 *Step 3) Adhesion of Bonding Pads to LIG* 5m

Note

Critical step:
Use caution and avoid touching the surface of the LIG material

- Using scissors, cut small 0.3 cm by 1.0 cm strips from the prepared polyester metal tape strips. One small subsection should be cut for each 3-electrode LIG sensor.
- Using steel tweezers, hold the metal tape and carefully remove the adhesive backing
- Carefully position the metal tape over one of the bonding pads on the printed LIG chip
- After coating the LIG reference electrode, the tweezers should be used to lightly squeeze the material and ensure good contact.
- Repeat the process for all three electrodes in the LIG chip (**Fig 2**)

Figure 2. Photo demonstrating adhesion of polyester metal tape strips to bonding pad of LIG design. Each bonding pad corresponds to the working, counter, or reference electrode.

Note

Critical step:
Care must be taken to ensure that the metal tape used to create bonding pads is not contacting other components of the chip, including other bonding pads.

4 *Step 4) Coating of LIG Reference Electrode* 5m

- The reference electrode may be coated with Ag/AgCl ink, or with a small 0.3 cm by 0.5 cm strip of polyester metal tape (as shown here)
- After coating the LIG reference electrode, the tweezers should be used to lightly squeeze the material and ensure good contact.
- If Ag/AgCl ink is used to coat the reference electrode, the material should be allowed to cure at room temperature for at least 15 min

5 *Step 5) Passivation of LIG Electrodes* 10m

- Use poly gel acrylic nail polish to passivate the electrodes between the bonding pad and the functional electrode area (**Fig 3A**).
- The passivation may cover a small portion of the polyester metal tape if used as reference electrode coating material (**Fig 3B**)

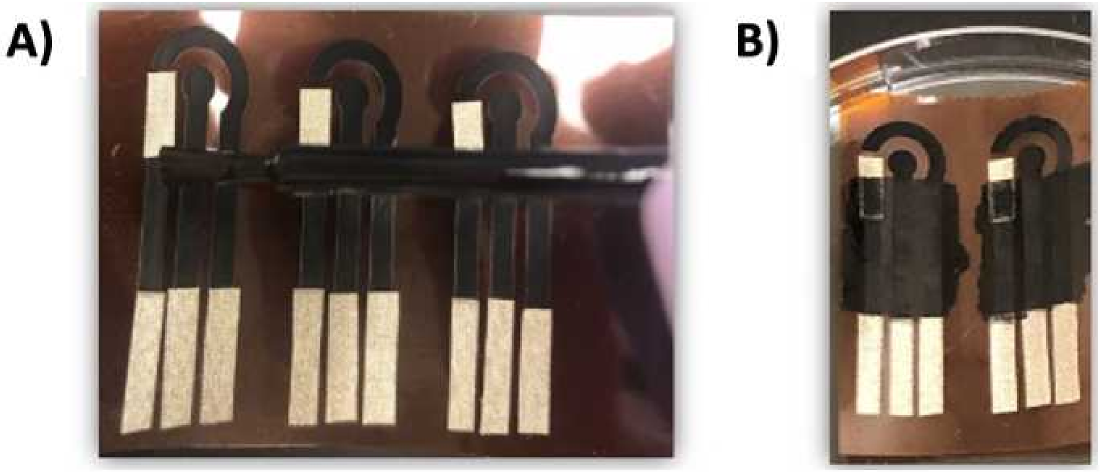


Figure 3. Passivation of LIG electrodes for creating functional electrode area and bonding pads.

6 *Step 6) Mounting LIG chip to PVC Base*

5m

- Using scissors, cut a strip of double sided tape and adhere to the back of the LIG material (**Fig 4A**).
- Mount the LIG material to the PVC base and use scissors to trim any excess tape.
- The final chip should have clearance with exposed, unmodified PVC which may be handled without touching the LIG or other coating materials (**Fig 4B**)
- The three electrode system is composed of working, counter, and reference electrodes. The passivation area and bonding pad are easily discernable (**Fig 4C**)

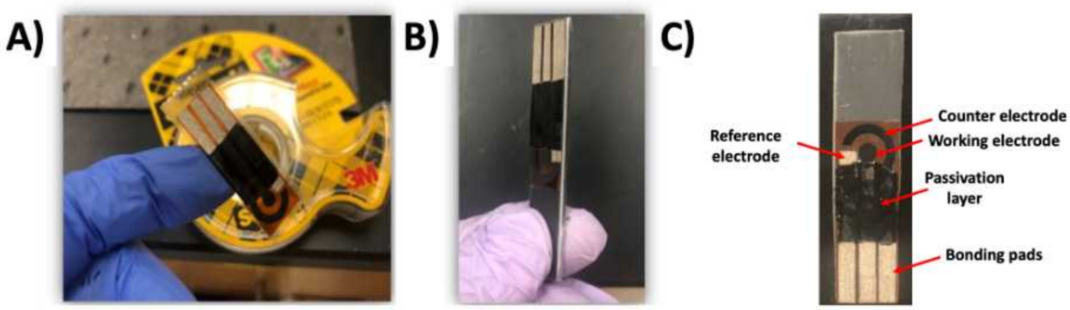


Figure 4. A) Cut a strip of double sided tape and adhere to back of LIG. **B)** LIG electrode mounted to PVC base. C) Example of completed LIG chip for plug-and-play with USB-A system. **C)** The final design is a plug-and-play 3 electrode LIG chip compatible with the USB-A adapter

7 *Step 7) Clean up laboratory* 10m

- Any excess polyamide, metal tape, or PVC sheet should be discarded in a labeled container on the counter.
- Turn off soldering iron, and clean station
- If storing electrodes, place in a sealed Petri dish with desiccant.

Protocol references

Moreira, G. *et al.* A capacitive laser-induced graphene based aptasensor for SARS-CoV-2 detection in human saliva. *PLoS One* **18**, e0290256 (2023).