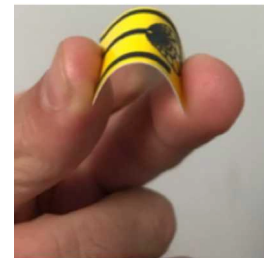


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🌐 Protocol L2.3 (LIG fabrication) V.1

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We use this protocol and it's working

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Abstract

This protocol describes the procedure for deposition of biotinylated aptamers on avidin-coated LIG electrodes. The complete process requires approximately 25 min to complete (**Fig 1**).

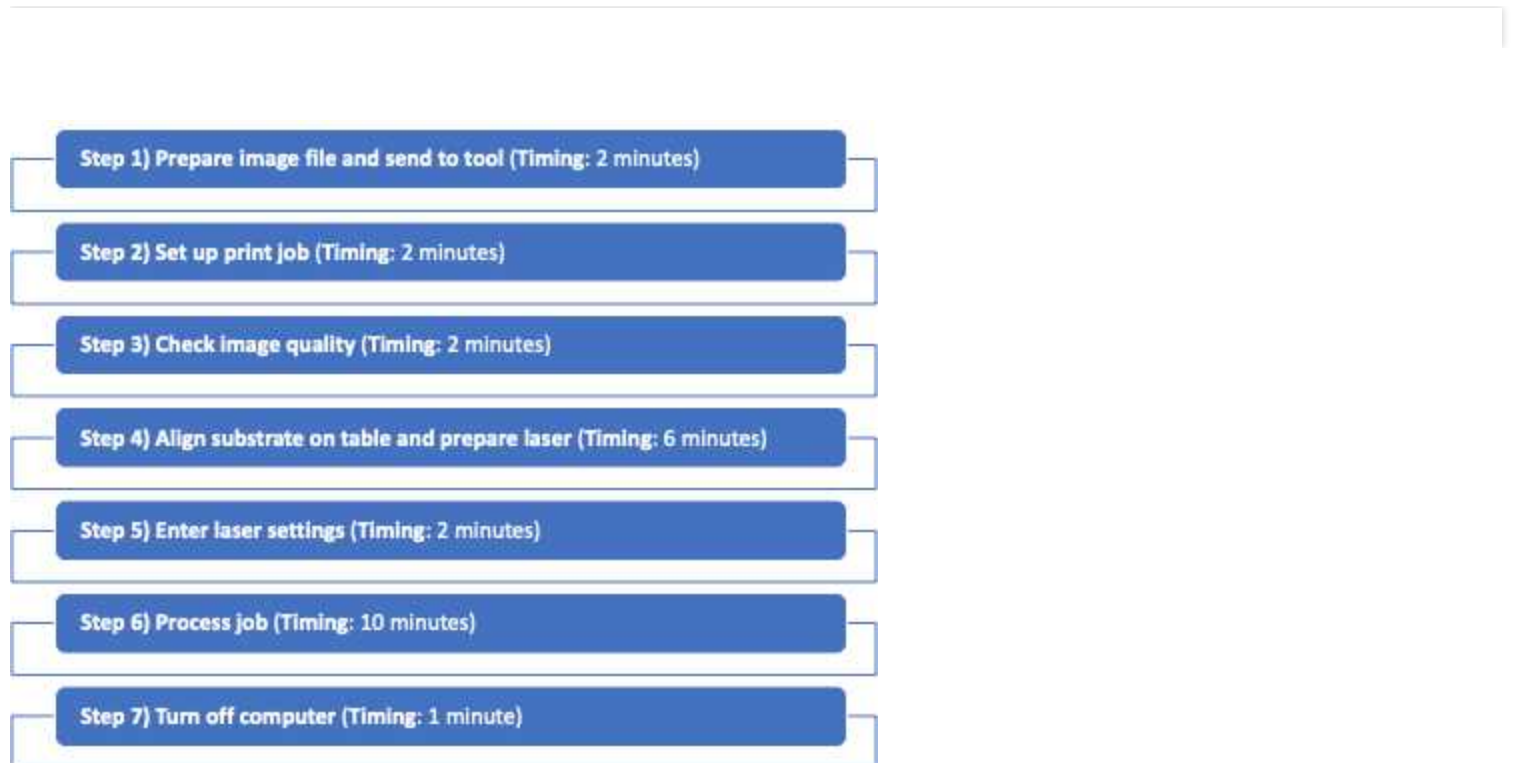


Figure 1. Process flow for aptamer loading protocol.



Materials

- Polyimide film (note thickness and type of polyimide)
 - o This procedure is for high heat temperature resistant adhesive tape (0.3 mm thick, 12X12 sheet) (<https://www.mcmaster.com/2271K3/>)
- Scissors
- Isopropyl alcohol
- Kim Wipes
- Tape
- Certified eyewear ([link](#))
- CorelDraw
- Universal Control Panel (UCP) ([link](#))
 - o If needed, download the software, and install on a Windows computer (will not work for Mac)
- Click on Support-Software Downloads
- Click on Download Drivers for VLS Platforms-UCP Installer

Safety warnings

! SAFETY

General laser safety



Caution: Laser safety

- EXPOSURE TO THE LASER MATERIAL PROCESSING BEAM CAN RESULT IN BURNS TO THE SKIN AND CAN CAUSE SEVERE EYE DAMAGE.
- Do not operate the laser system if the view port is damaged, with any of the doors removed, or if any of the safety interlocks are defeated.
- This device contains a visible Red Laser Pointer (Class 2) to aid in positioning material to be cut, marked, or engraved. DO NOT LOOK DIRECTLY INTO THE RED LASER BEAM OR USE A REFLECTIVE SURFACE TO REDIRECT OR VIEW THE RED LASER BEAM. Never attempt to view the red laser beam using optical instruments such as binoculars or microscopes.
- The access door(s) are safety interlocked, which will prevent the CO₂ laser beam from firing when the access door(s) are opened. The Red Laser Pointer is NOT safety interlocked and can be automatically activated with the door(s) either open or closed.
- DO NOT OPERATE THE LASER SYSTEM IF ANY SAFETY FEATURES HAVE BEEN MODIFIED, DISABLED OR REMOVED.
- Details on laser safety may be found at the following website ([link](https://www.ulsinc.com/resources/laser-safety))
<https://www.ulsinc.com/resources/laser-safety>

Eye protection

- WHEN USING THE OPTIONAL **CLASS 4 CONVERSION MODULE**, PROPER EYEWEAR MUST BE USED AT ALL TIMES WHEN THE BEAM INDICATOR IS ILLUMINATED ON THE CLASS 4 CONTROL PANEL.
- The intense light that appears during the laser engraving, marking, or cutting process is the product of material combustion or vaporization. DO NOT STARE AT THIS INTENSE LIGHT FOR LONG PERIODS OF TIME OR VIEW DIRECTLY WITH OPTICAL INSTRUMENTS SUCH AS BINOCULARS OR MICROSCOPES.
- Eyewear must be properly certified for use with and protection from all material processing laser wavelengths in use (10.6-micron, 9.3 micron, and/or 1.06 micron) and be at least optical density 5+.

Skin

- The laser will burn skin so always avoid direct contact
- If replacing a laser head turn all power off before handling the laser system
- When adjusting laser focus, always use gloves

Fumes/aerosols



Caution: Fumes and aerosols may be hazardous

- Ensure that the built-in fan for the engraver is functioning (check monthly)
- A properly configured, installed, maintained, and operational particulate and fume exhaust system is mandatory when operating the laser system. Fumes and smoke from laser material processing must be extracted from the system and filtered or exhausted outside.
- **SOME MATERIALS, WHEN ENGRAVED, MARKED OR CUT WITH A LASER, CAN PRODUCE TOXIC AND CORROSIVE FUMES.** Obtain the Material Safety Data Sheet (MSDS) from the manufacturer of each material to be processed. The MSDS discloses all the hazards when handling or processing a particular material. DISCONTINUE processing any materials that cause chemical deterioration of the laser system such as rust, metal etching or pitting, peeling paint, etc. Damage to the laser system from corrosive fumes is NOT covered under warranty.
- There is not information in the MSDS for polyimide about harmful effects of inhaling fumes, but safety should be taken to avoid exposure through inhalation.
- The backing material plays a major role in the amount of vapor/smoke produced and this should be carefully monitored when changing materials.
- If a hood is not available, a PVC pipe may be fixed to the fan on the engraver and the exhaust can be pulled into a chemical hood.

Heat and Flammable materials



Caution: Heat and Flammable Materials



- Never operate the laser system without constant supervision of all cutting, marking, and engraving processes. Exposure to the laser beam may cause ignition of combustible materials which can lead to a fire. A properly maintained fire extinguisher should be always kept on hand.
- A remote connection to the system across a network does not negate the need for constant supervision of all cutting, marking, and engraving processes.
- Never leave materials in the laser system after processing. Always remove all material, including scrap material, from the machine after use. Scrap material left in the laser system, including materials that collect in the removable **Flow Through Cutting Table**, can be a fire hazard. Materials can be hot after processing so caution should be used when handling material immediately after processing. A properly maintained fire extinguisher should be always kept on hand.

Disposal

- Non-modified polyimide samples should be discarded in the bins marked “Kapton film” next to the Laser system

Before start

Critical step:

- Wear eyewear protection at all time
- Wear gloves to avoid transferring fingerprints to the Kapton tape

LIG on polyimide film

25m

1 Step 1) Prepare image file for processing and send to tool

5m

- Verify that the Universal Control Panel (UCP) is running in the taskbar by looking for the square red icon with a diamond (**Fig 1A**)
- Using CorelDraw, Open the image called "LIG working electrode_triple_COVID" and press Cntrl+P and select VLSprinter (VLS3.50/60DT) (**Fig 1B**). This electrode has the following dimensions, verified with the digital caliper under the inspection microscope

Electrode diameter= 3.0 mm (diameter)

Stem area= 14.3 mm X 2.0 mm (length X width)

Bonding pad area= 2.9 mm X 2.5 mm (length X width)

Feature size of "Clemson University" = 60 μ m (4-point Arial font in CorelDraw)

- Select properties in print screen and change the following settings (**Fig 1C**)
- Click Apply, OK (**Fig 1D**)
- Click Print to send job to laser

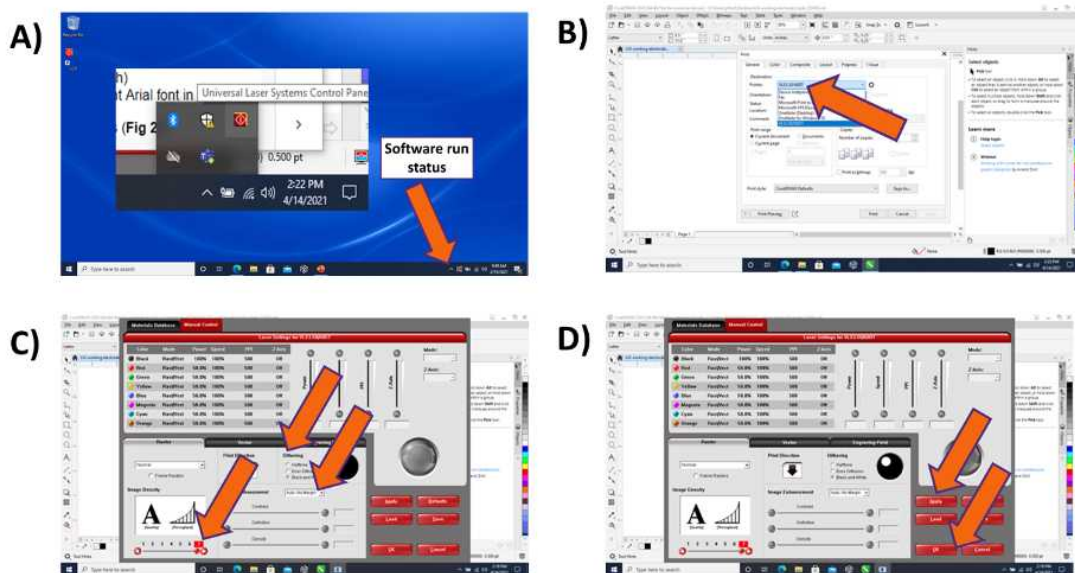


Figure 1. Prepare image and send print job to instrument **A)** Verify UCP is running. **B)** Open image in CorelDraw and press Cntrl+P. Then make sure the correct printer is selected (VLS3). **C)** Select properties in the CorelDraw print screen and change the parameters for image density, image enhancement, and dithering **D)** Click apply, OK. Then click print in the CorelDraw panel to send the job to UCP software.

- 1.1 1m
- Select properties in print screen and change the following settings (**Fig 1C**)

Image density=7

Image enhancement=Auto No Margin

Dithering=black and white

2 Step 2) Set up print job

5m

- Launch UCP software (**Fig 2A**).
- Select the System Tab and verify that the Auto Z box is checked (**Fig 2B**).
- Return to Viewer Tab and turn on the instrument or turn on manually (**Fig 2C**).
- Alternatively, turn on the instrument manually (**Fig 2D**).

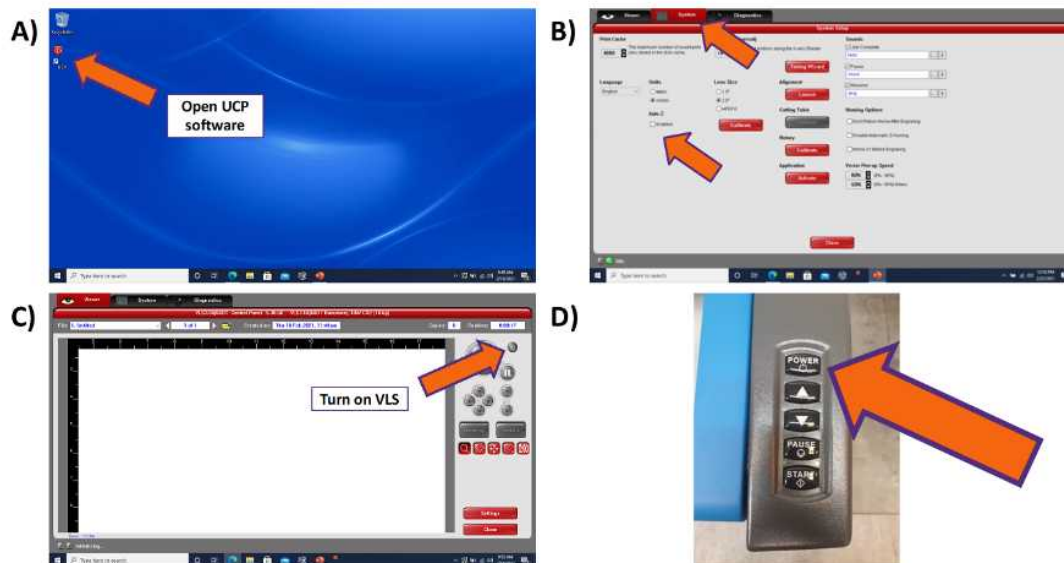


Figure 2. Quality check and printer setup. **A)** Open UCP software. **B)** Ensure Auto Z box is checked in Systems tab and then turn on VLS instrument with software **C)** Click on the Viewer tab. Turn on instrument using UCP software, or **D)** Alternatively turn on instrument manually.

3 Step 3) Check image quality

2m

- After sending the print job to UCP, check image for quality.
- If using color, ensure that all features are transferred correctly.

4 Step 4) Align substrate on table and prepare laser

5m

- Prepare a 4" by 4" (10cm X 10 cm) sample of Kapton film
- Tape the sample onto the table (see **Fig 3A**)
- Ensure that the workpiece is flat. Any bends must be accounted for in the "Z axis setting" (this advanced feature is not discussed in this protocol)

Note: If the workpiece is not flat, use the heat press (see protocol HP1.1)

- Place a few drops of isopropyl alcohol on a Kimwipe and clean the surface.

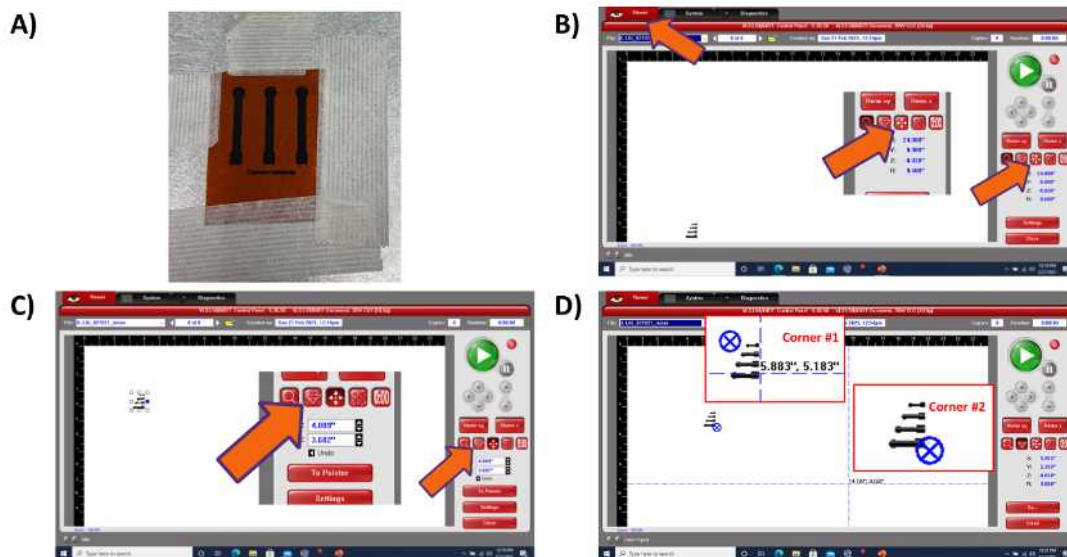


Figure 3. Image processing. **A)** Tape sample onto table. **B)** Scale is corrected (using the original image software). The scalebar on the instrument and the software should be used as a ruler to ensure the correct scale is used. **C)** Use “move to position” feature to check corners of the carving space. **D)** Move the laser to at least two corners of the image to check the IR laser in the instrument is aligned on the sample (and not the tape)

Critical step:

DO NOT PUT ALCOHOL DIRECTLY ONTO THE SAMPLE WHEN IT IS INSIDE THE INSTRUMENT. NO LIQUIDS IN THE INSTRUMENT AT ANY TIME. THE ALCOHOL SHOULD BE SPAYED ONTO A **KIMWIPE**, NOT THE SAMPLE

5 Step 5) Enter laser settings

- Select “settings” in the menu near the bottom right (top arrow, **Fig 4A**).
- Be sure that the “move laser to” button is NOT engaged, this will close the “Settings” button (bottom arrow, **Fig 4A**).
- Choose the materials database in the tab section (top left) (**Fig 4B**).
- Choose DuPont Kapton tape as a starting point and click apply then Click “OK”(**Fig 4C**)
- Select “settings” in the menu near the bottom right (top arrow, **Fig 4A**).
- Click on “Manual Control” in the tab section (top left) (**Fig 4D**).
- Set the power, speed and PPI as shown below (**Fig 4E**).

Power=40

Speed=75

PPI=1000

Zaxis=0.005'

2m

- Click “SET”, then Click “Apply” then Click “OK”(Fig 4F)
- After returning to the print screen, return to the manual control screen to be sure the settings were saved

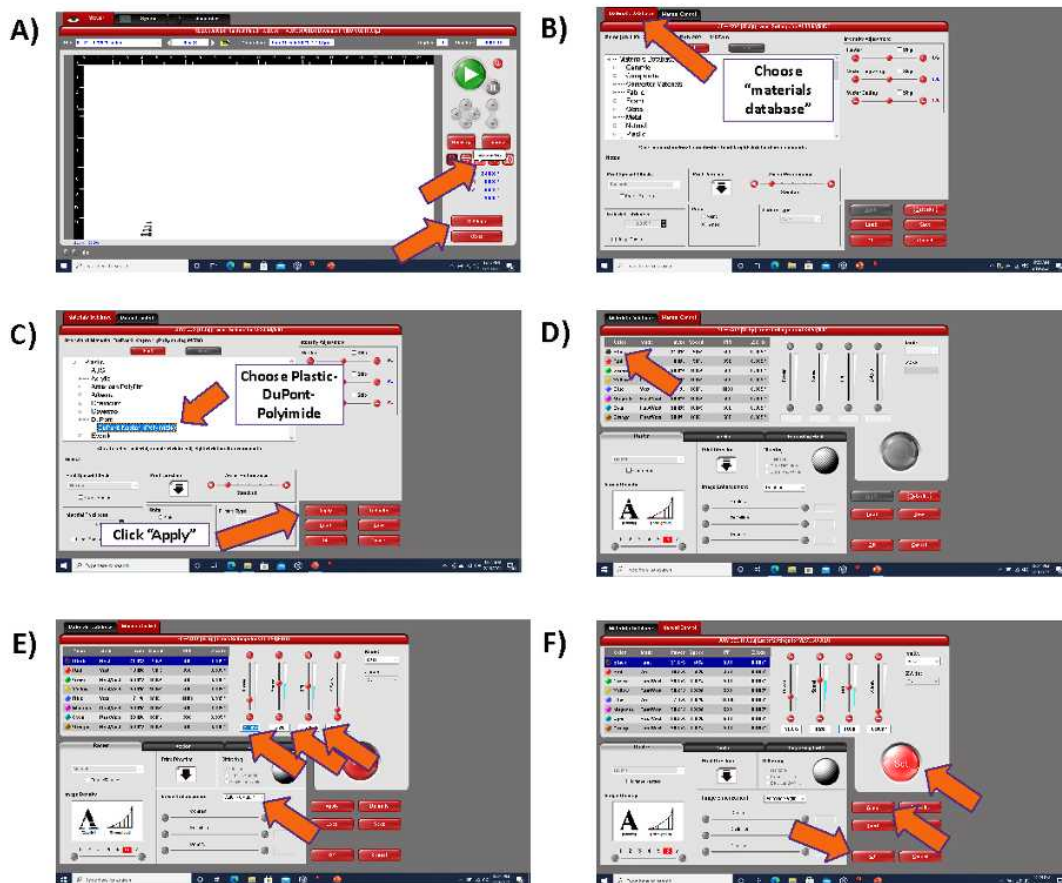


Figure 4. **A)** Choose “Settings” tab. **B)** Choose the “Materials Database” tab. **C)** Choose DuPont Kapton tape and click apply. **D)** Click on “Manual Control”. **E)** Set the power, speed, and PPI according to the matrix design. **F)** Click “SET”, then Click “Apply” then Click “OK”.

6 Step 6) Process job, pause if necessary

- Press the go button to start the job (Fig 5A).
- The time for the job is shown in the top right area of the screen (Fig 5B).
- Do not directly look at the plasma during the process, a photo is below if you are curious (Fig 5C).
- The job can be paused using the software or the manual control (Fig 5D).

Pushing the green button will restart job from the beginning

Pushing the resume button will continue job where it was paused

10m

- Do not open the hood during a job unless it is an emergency

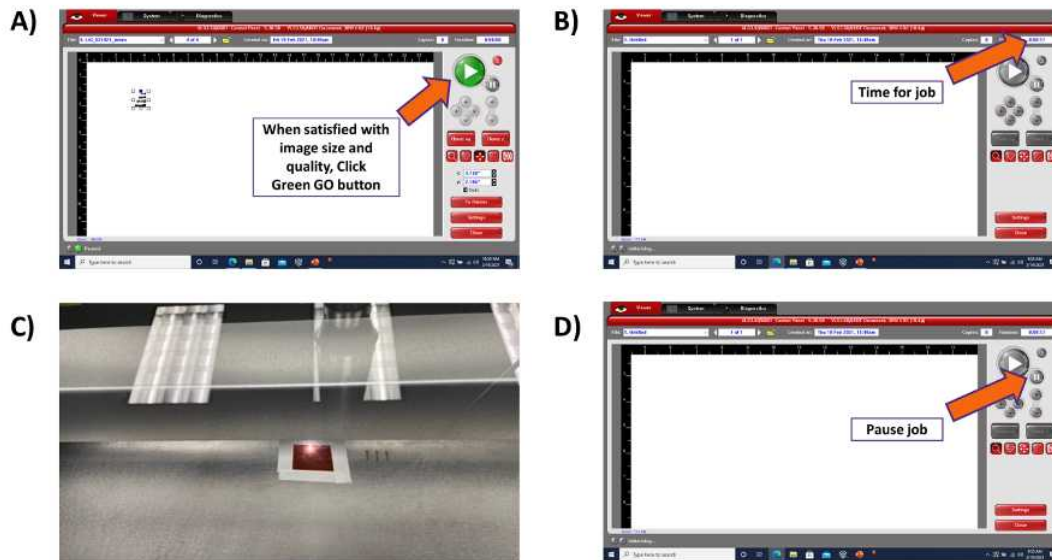


Figure 5. Pausing a job or stopping a job. **A)** Green button will restart the job from the beginning. **B)** Time of the job is shown in the top right corner. **C)** Photo of laser carbonizing Kapton film. Unless needed, do not open the hood to stop a job, this is an emergency feature and can cause the sample to be ruined. **D)** Pause button should be used if necessary but will likely cause irregularities in the sample.

Note: See protocol LQC for quality control analysis of electrodes

7 Step 7) Turn off computer

- The auto-power function is disabled on the computer, PLEASE TURN COMPUTER OFF WHEN JOB IS COMPLETE

8 Step 8) Data Management

File naming: For saving all versions of this protocol, use the following file structure:

LIG1.1_091721.ai

File storage: Store all methods in the Desktop folder.

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

1m