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3D Electrogenetically Controlled Gene Expression Bioelectrode (ECOGEB) Process Development: Using Two-Photon Polymerization Fabrication for Metal-Doped Resin and Pyrolysis

by Jose A. Wippold, Jessica Liba, Pranav Ampani, and Ana Cohen

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3D Electrogenetically Controlled Gene Expression Bioelectrode (ECOGEB) Process Development: Using Two-Photon Polymerization Fabrication for Metal-Doped Resin and Pyrolysis

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1. Introduction

Micro- and nanofabrication play a crucial role in defense technology and enable the development of advanced materials and systems. These techniques allow for the creation of miniaturized devices (e.g., sensors, antennas, and electronics) that enhance surveillance, communication, and navigation capabilities. Nanomaterials also improve armor, explosives, and propulsion systems. Through this work, we aim to marry the topics of bioelectronics, micro- and nanofabrication, and capabilities development (Figure 1). This report focuses on the engineering necessitated by the overall project goals in developing the methods for fabrication process development.

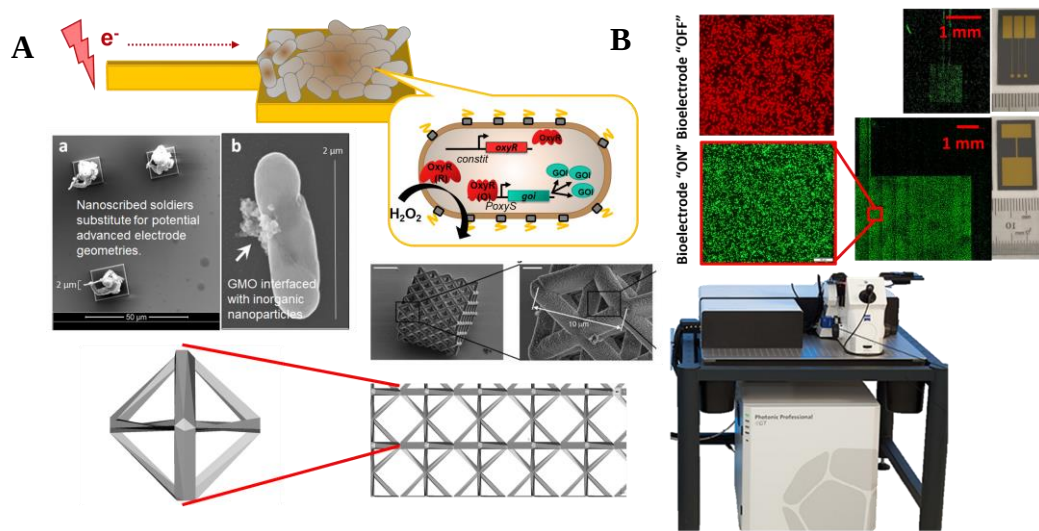


Figure 1. Overview of 3D Electrogenetically Controlled Gene Expression Bioelectrode (ECOGEB) project. A) Schematic of a bioelectrode with example nanofabricated structure. B) Application use-case example of the bioelectrode demonstration “on” and “off” states. Nanoscribe’s Photonic Professional GT at the bottom.

Additionally, micro- and nanofabrication facilitate the production of secure and reliable components, reducing the risk of cyber threats and component failure. By leveraging these technologies, defense agencies can create innovative solutions to stay ahead of emerging threats and protect national security. This drives innovation and modernization efforts.

Within the U.S. Army Combat Capabilities Development Command Army Research Laboratory Specialty Electronic Materials and Sensors Cleanroom (SEMASC), manufacturing nano- and microscale features requires a set of very precise tools and techniques. The two-photon polymerization (2PP) process is used to fabricate structures of about 220 nm in resolution, which is smaller than the thickness of a human hair (Figure 2). Using the Nanoscribe Photonic Professional

GT 3D printer, the 2PP process can be used to manufacture complex, yet small, structures. These structures are made with the IP-S photoresist and can be pyrolyzed after printing to create a carbonized and conductive structure used to make bioelectrodes.¹ The introduction of metal additives into the IP-S photoresist is also investigated with the goal of increasing the conductivity of such structures.^{2,3} This body of work marks the first step in driving nanobioelectronic development at DEVCOM Army Research Laboratory (Figure 3).

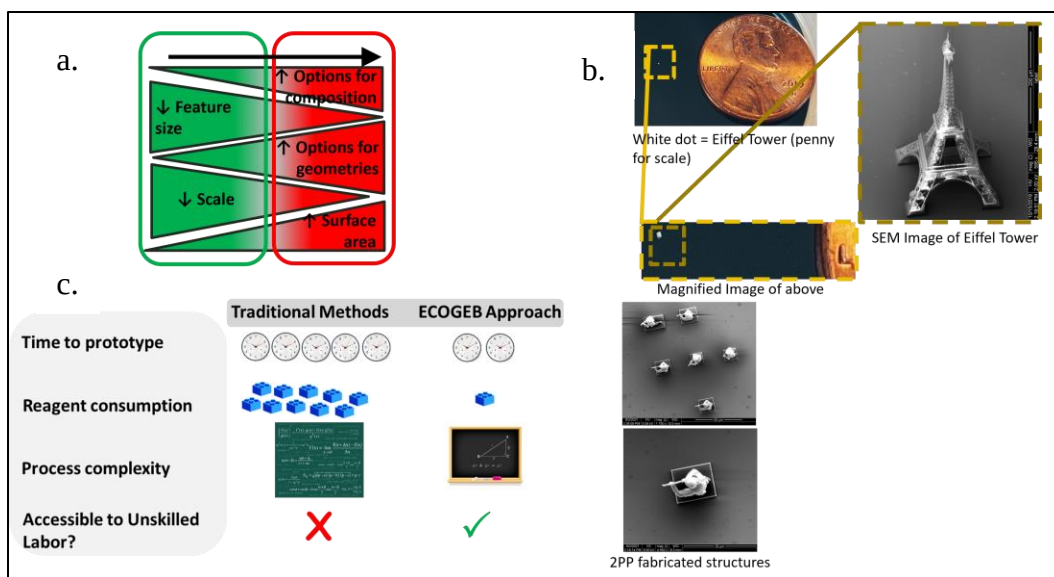


Figure 2. Pros and cons of 3D ECOGEB approach. a) Pros and cons of 2PP. b) Resolution capabilities of Nanoscribe's 2PP cleanroom tool. c) Benchmarking of prototyping time, reagent consumption, process complexity, and accessibility.

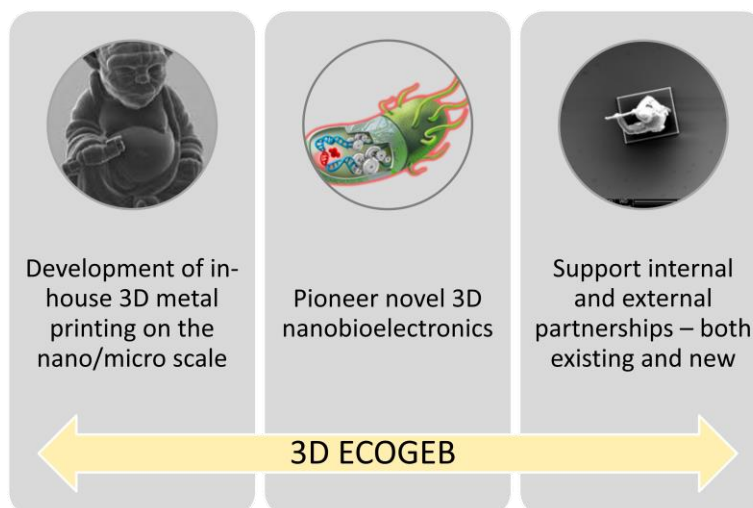


Figure 3. 3D ECOGEB project aims championed at ARL.

2. Experimental and Results

2.1 CAD

SolidWorks was used as the primary CAD software for the entirety of this project. Initially, different lattice structures were made from the bottom-up, requiring a new model every time there was a change in design criteria and parameters. As the project progressed, a template was created using the built-in “Equations” tool (Figure 4) to control the various parameters used to modify the lattice and its supporting structures. These values change the size and shape of the supporting coils and the size and number of unit cells in the lattice structure. The template can also be used to change various other dimensions used in the CAD model; however, that would be unnecessary since nearly the entirety of the dimensions use the values under “Global Variables.” Thus, rapid prototyping was enabled as it was now possible to make many different models by changing one or two values within the CAD file itself.

The creation of the 3D model started with the unit cell itself, which was then patterned to meet the requirements of the use-case criteria. After the lattice structure was created, the supporting structures were added. They included a square base directly underneath the lattice structure, along with coils attaching it to another square base that would print directly onto the substrates. This can be followed in Figure 4, which includes all the commands used to create each part of the model.

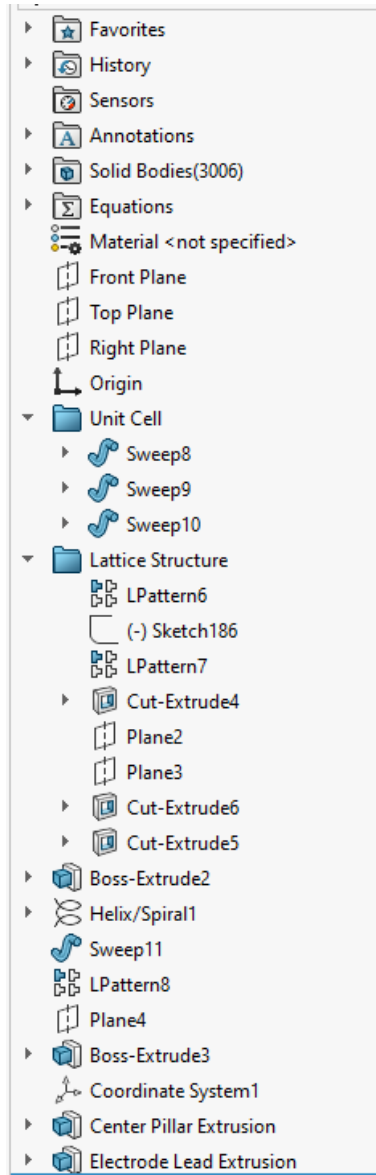


Figure 4. Feature tree of CAD model used in prints.

As extra supporting features, a center pillar and electrode leads were also added. The center pillar was essential for the larger structures, which was anything above $500 \times 500 \times 500 \mu\text{m}$. The electrode leads were included to allow for the testing of conductivity of the structures without the potential of damaging the fabricated lattice structure of interest.

Figure 5 is an example of the Equations Tool used in this work. As aforementioned, this functionality is used to change the various parameters and dimensions used to model the lattice structure. To navigate to the Equations Tool, one would need to right-click the “Equations” item in the Feature Browser, and then select “Manage Equations . . .” A window will pop up showing all of the Global Variables, Features,

and Equations used in the model. The entirety of the dimensions in the model use one or more of the Global Variables, with the exception of the coil revolutions and the electrode pad dimensions, which remain constant from design to design. Following Figure 6 step-by-step will allow you to change the “unit thickness” from 0.01 to 0.0167 mm, and the “coil radius” from 0.05 to 0.075 mm. These two changes result in the entire model shifting uniformly. This enables the structure to keep the original geometrical shape despite the adjustments made to the other dimensions in different parts of the structure.

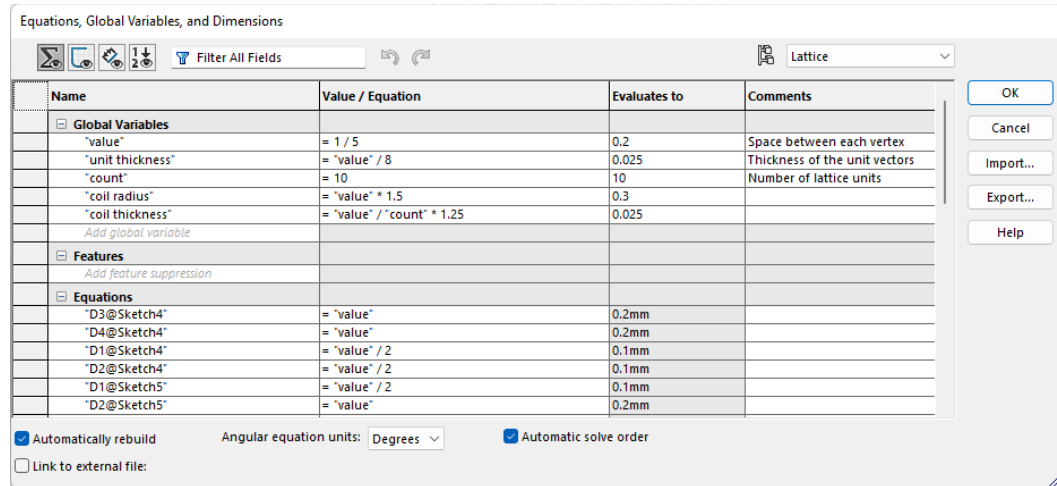


Figure 5. The equations tools in SolidWorks.

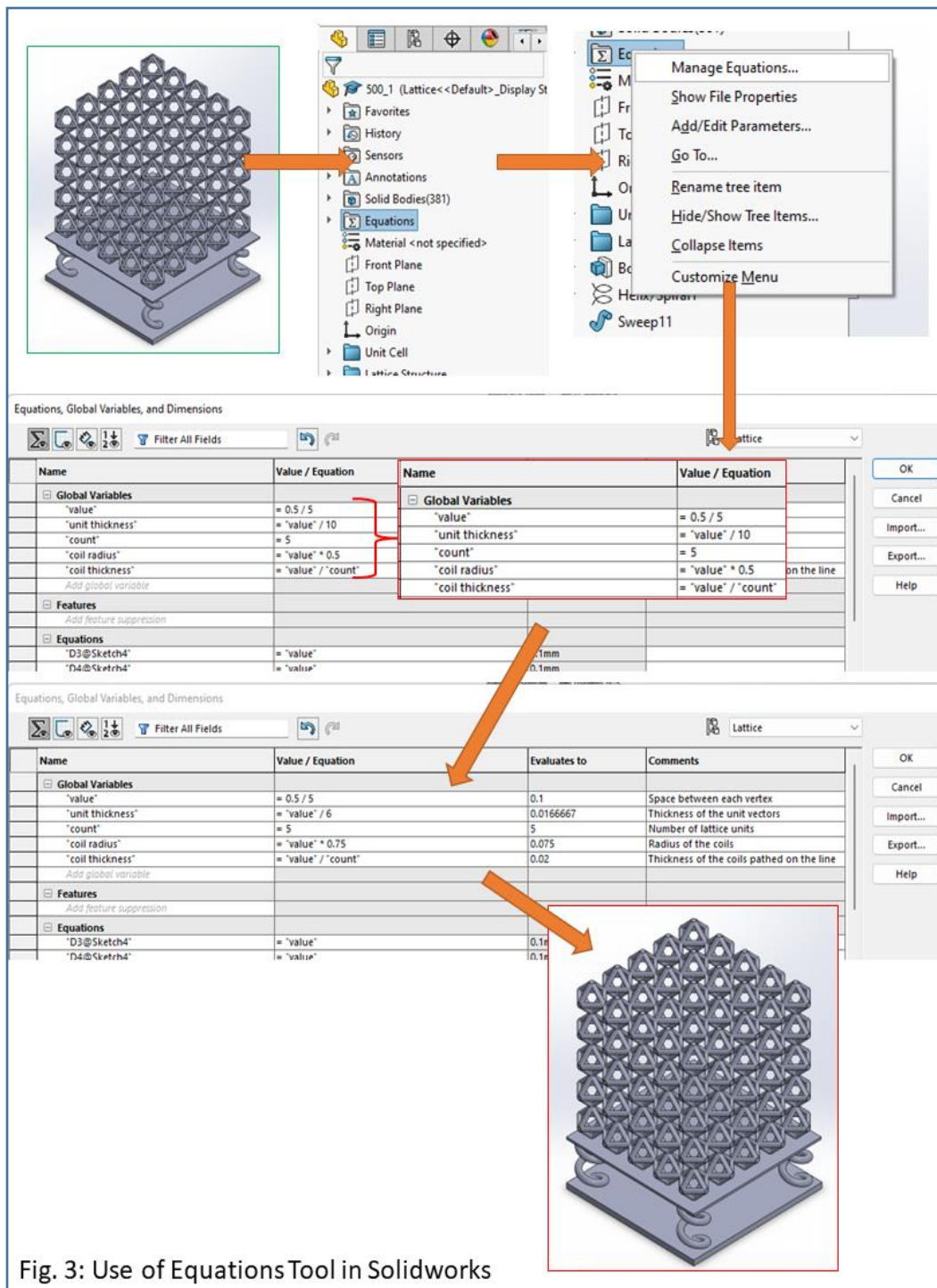


Fig. 3: Use of EquationsTool in Solidworks

Figure 6. Stepwise usage of the equations tools in SolidWorks to facilitate design process.

2.2 2PP: Nanoscribe Photonics GT

The Nanoscribe Photonic Professional GT was used as the fabrication equipment for the entirety of this work. In brief, it uses 2PP to polymerize a photoresist into structures dictated through a proprietary coding language that is fed user CAD designs.⁴⁻⁶ The photoresist used for this project was IP-S, which was used in tandem with the 25× objective lens provided by Nanoscribe.

DeScribe is the program used to convert STL files (output from SolidWorks) into code for the Nanoscribe (Figure 7). In brief, DeScribe takes an STL and then prompts the user to input several parameters to calculate the most efficient writing process (i.e., a combination of stage movements and laser actuations). The main parameters that impacted this project were the “blocking parameters.” This included block size and offset for the X, Y, Z directions, as well as shear angle and print pattern (Figure 7). These parameters are highly impactful, especially as the size of the structure increases. Typically, the blocking parameters had been set to the default values for the smaller structures (e.g., $300 \times 300 \times 300 \mu\text{m}$ and smaller) allowing Nanoscribe to print the structure in one field of view, thus eliminating the need for mechanical stage movements and saving time. However, any larger structures require the blocking parameters to be modified manually. For example, for a $500 \times 500 \times 500 \mu\text{m}$ structure, a block size of $250 \times 250 \mu\text{m}$ in the X and Y directions was used along with a modified Z-block height set to a point preventing any floating structures in the photoresist.

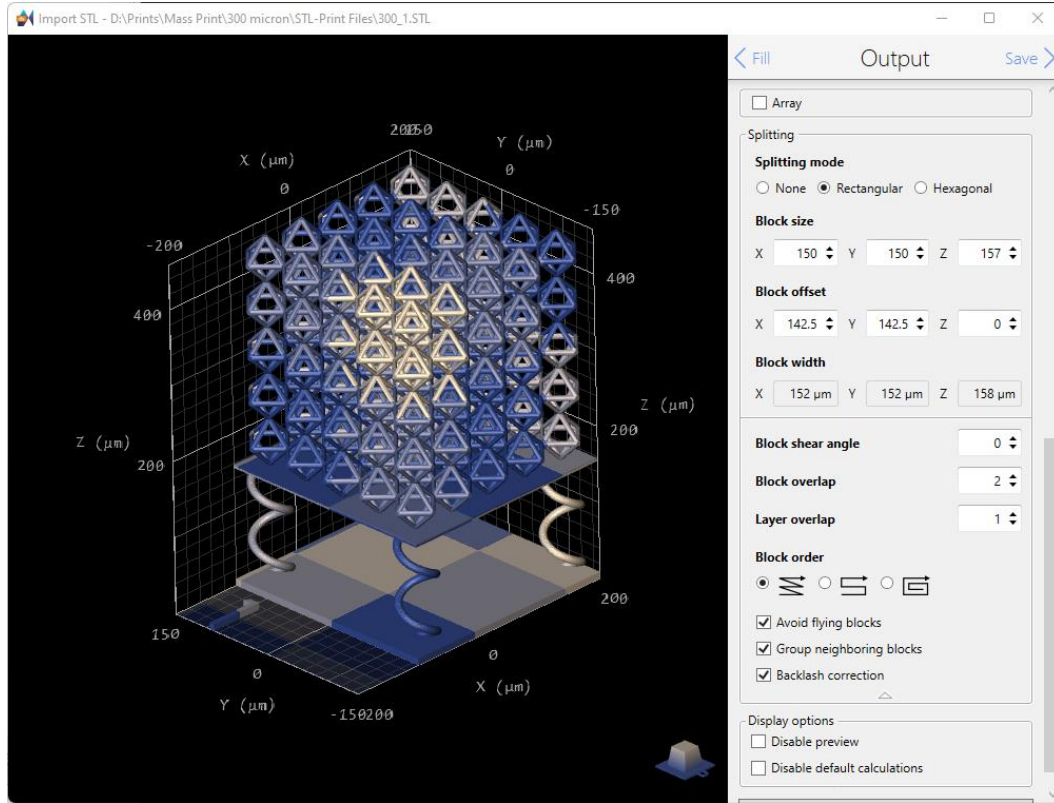


Figure 7. DeScribe STL processing window that displays the splitting parameters and options.

2.3 Troubleshooting for DeScribe Parameters

The parameters used for the various prints created during this project varied depending on several factors. First, the most important factor was the size of the structure itself. Structures up to $300 \times 300 \times 300 \mu\text{m}$ in size can be printed in one block in the X–Y plane; however, it would necessitate two to three blocks in the Z-direction (depending on the height of the coils used) to support the structure. The best method for printing larger structures was found to be printing them with blocks that span a 1/4 to 1/2 of the structure in the X–Y plane, ensuring each block splits at a good position (i.e., avoiding critical geometry) where there are no floating parts of the structure between the galvo movements of the Z-direction.

Second, the coil height was another consequential factor impacted by the DeScribe blocking parameters. The height of the coil impacts the employed Z-offset. Usually, a user would want the Z-offset positioned in a way that would prevent the small plate directly below the lattice structure from floating for an extended period (e.g., $>5 \text{ s}$). One method to overcome this floating was to implement the offset halfway up the small plate. In turn, this results in the transition from coils to lattice array to

be more solid compared with a process resulting in a block including a portion of the coil with the lattice array.

The basic procedure for fabrication using the Nanoscribe GT is as follows:

1. Procure diced silicon oxide substrates (sized $\sim 1 \times 1$ inch).
2. Soak each substrate in acetone for 15 min.
3. Wash off each substrate with isopropyl alcohol (IPA).
4. Use the PVA TePla plasma furnace to further clean the surface of the substrates.
 - a. Vent the furnace before attempting to open it.
 - b. Place the substrates onto one of the racks within.
 - c. Close the furnace door and select “Load Recipe.”
 - i. Recipe name: RF-STRIP-5MIN
 - d. Once the recipe is loaded, select “Run” and select a batch number (if needed).
 - e. Wait for the furnace to complete the recipe (takes $\sim 6\text{--}7$ min).
 - f. Remove the substrates from the furnace and close the door.
 - g. Select “Idle Vac” on the furnace.
5. Take the substrates to the Nanoscribe and put them in the respective holder.
 - a. For multiple substrates, use the 3×3 diced substrate holder.
6. Tape down the substrates on the sample holder to secure them.
7. Add a drop of IP-S onto each substrate.
 - a. Make sure that the spatula from the bottle does not touch the surface of the substrates to prevent contamination of the resist supply.
8. On Nanoscribe computer, and in the Nanoscribe software, select “Exchange Holder.”
9. Once prompted, select the correct holder but do not hit “Exchange.”
10. Check the objective lens in the Nanoscribe. If it is not the $25\times$, remove the current objective lens and replace the lens cap on the Nanoscribe.
11. Rotate the objective disc until you have access to the $25\times$ slot and screw in the $25\times$ objective lens. Rotate the lens back to the center position.
12. Insert the sample holder upside down into the Nanoscribe.
13. Go back to the computer and select “Exchange.” The Nanoscribe will now conduct a series of movements to calibrate and position itself for the samples.
14. Select “Approach Sample.” This will simply move the objective lens into the photoresist of one of the samples on the holder.
15. Next, select “Load Job.” Select the job file from the location it is stored.
16. Click on “Run.” The Nanoscribe will now start printing. Stay for a few minutes after the print has started to observe the printer in action. This will

- allow confirmation of the print sticking to the surface of the substrate or observation of any issues with the printer during the job.
17. Once the print has finished, select “Exchange Holder.”
 18. Remove the sample holder from the Nanoscribe and place it on a flat surface.
 19. Carefully begin removing the tape on each of the substrates.
 20. Go to a fume hood and prepare two to three beakers. Two of the beakers will be filled with propylene glycol monomethyl ether acetate (PGMEA), which is the developer for the photoresist. The other beaker will be filled with IPA.
 21. Bring over the two vertical sample holders from the Nanoscribe desk.
 22. Bring the sample holder over to the fume hood.
 23. Place one substrate in each of the vertical sample holders and place both in the two beakers of PGMEA. Make note of which one was placed first. Leave them in the beakers for 7 min or until the IP-S has completely developed off the surface of the substrate.
 24. Afterward, take the first substrate and place it in the IPA beaker for 2 min.
 25. Once that substrate is done, place the next substrate in the IPA.
 26. Repeat step Nos. 23–25 until all the substrates have been developed and postprocessed.
 27. Now the samples are all ready to be observed under a microscope or the scanning electron microscope (SEM).

3. Observations

After processing each model, quality control was conducted using a Leica microscope located in SEMASC and a SEM housed in the Biotechnologies Laboratories at ARL. The LEICA microscope was used to conduct preliminary analyses of the structures after printing as well as postpyrolysis. Figures 8–10 show a variant of the model printed with the IP-S photoresist used to increase the coil height and the number of revolutions it had.

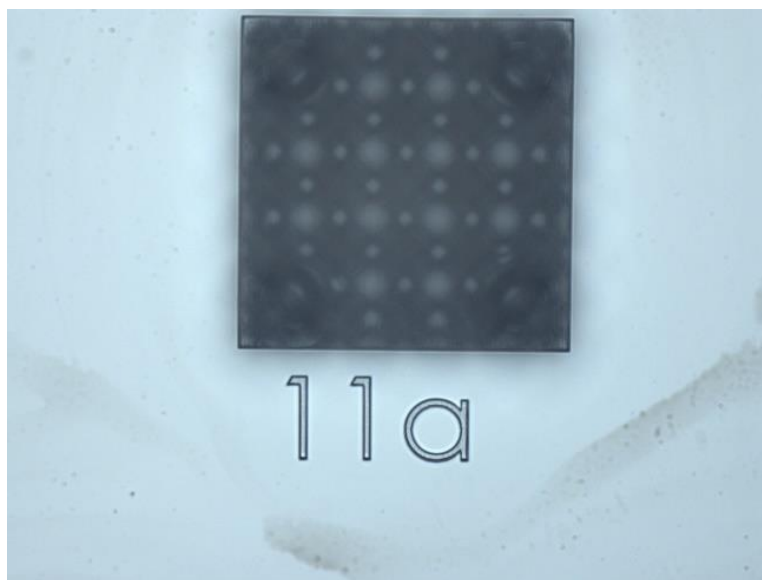


Figure 8. 50× image of variant 11a containing a $300 \times 300 \times 300 \mu\text{m}$ lattice structure in a $5 \times 5 \times 5$ -unit cell array.

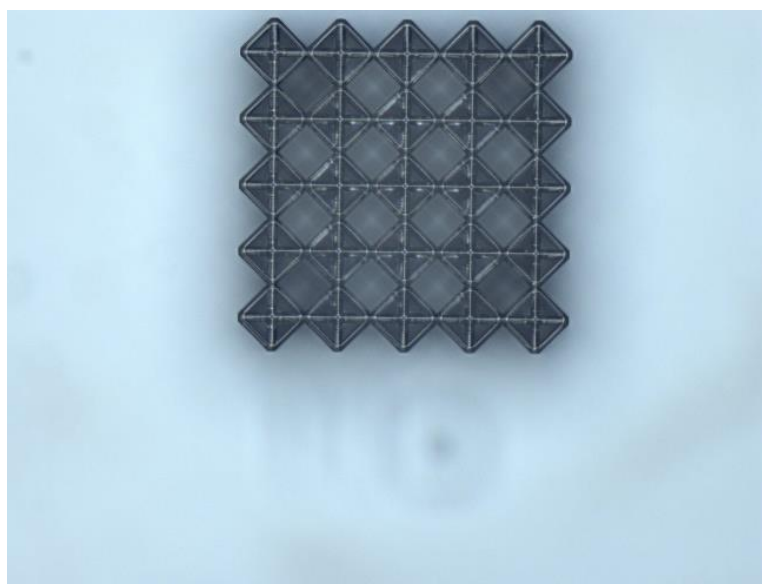


Figure 9. 50× image of the top plane of variant 11a.

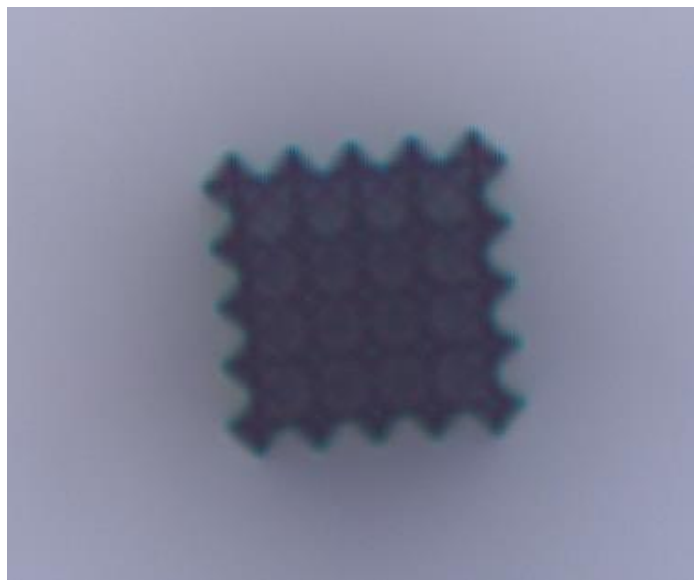


Figure 10. 100× image of microfabricated structure postpyrolysis.

The SEM provides a much more detailed observation of the lattice structures, especially after pyrolysis. While still providing a micrograph of the entire structure, such as in Figure 11, SEM imaging was also able to zoom to a granular detail beyond what a standard Leica microscope is capable of capturing. This enables the ability to interrogate the minute differences between the various types of coils and sizes. The best example of this occurred when observing the larger structures we printed, such as $1\text{-} \times 1\text{-} \times 1\text{-mm}$ arrays. Although the block sizes used in DeScribe were set up to make sure there were no floating blocks, it was still unknown how the increased print time would affect the print physically. Figure 12 shows the observations made with the aid of the SEM. The conducted imaging demonstrated how the blocking used created points in the structure where the unit cells were not aligned. This could potentially cause issues during pyrolysis and for subsequent printing with a copper (Cu) acrylate IP-S mixture. This introduces a new variable to investigate when it comes to printing larger structures.

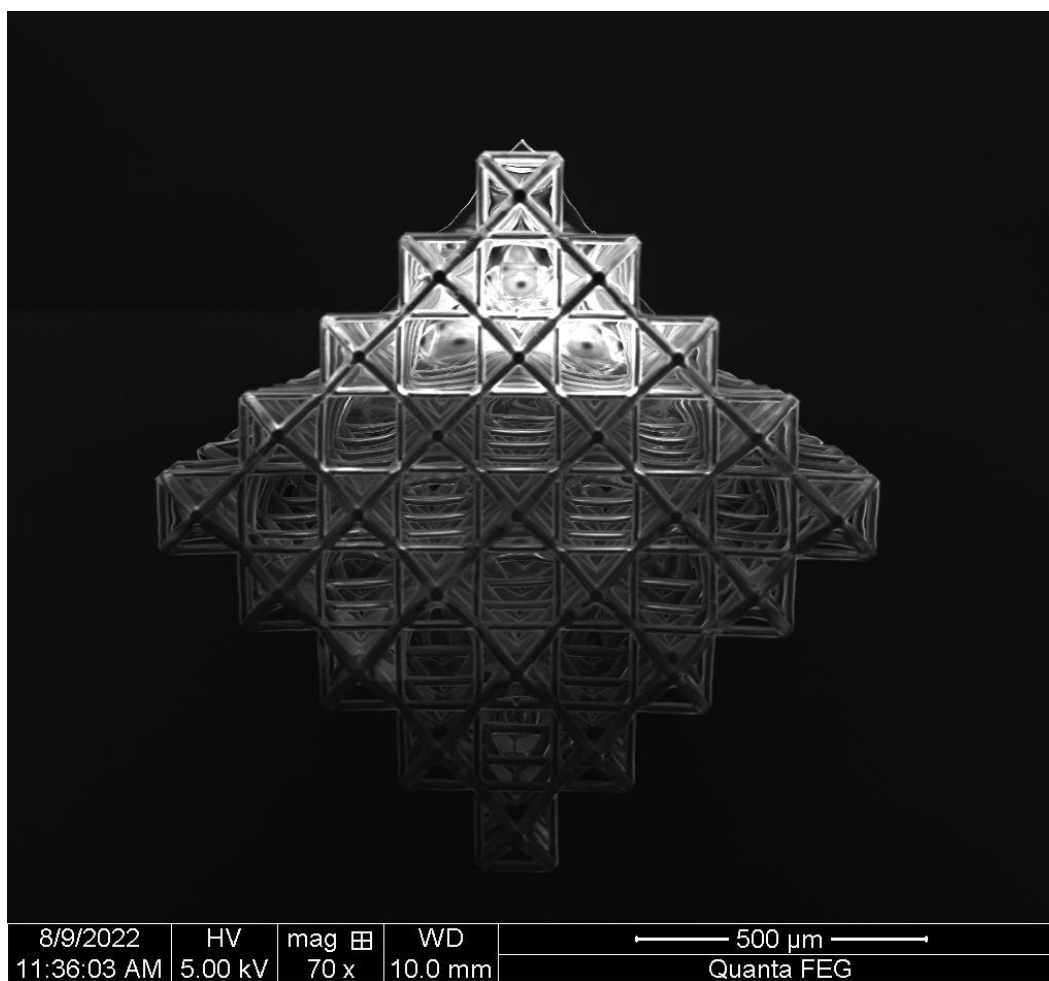


Figure 11. SEM image of the 1000- × 1000- × 1000-μm structure.

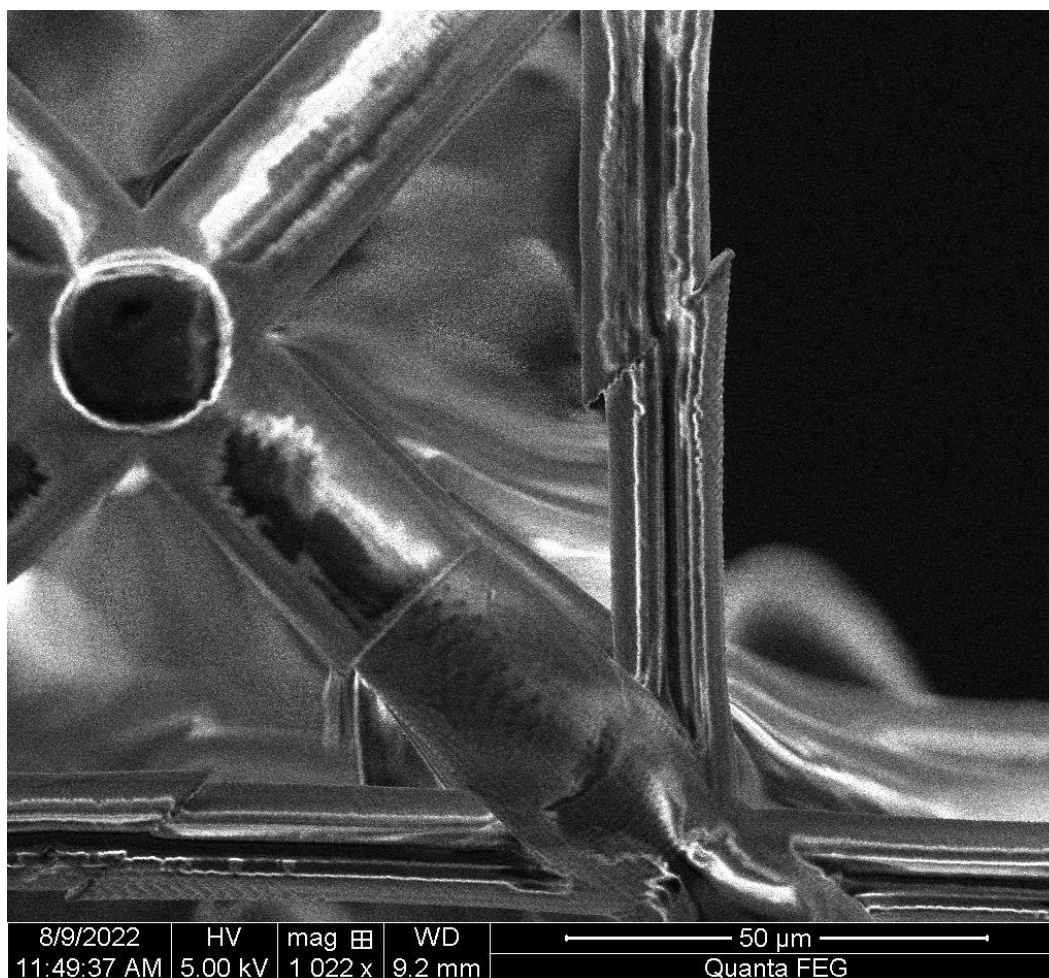


Figure 12. SEM image of the 1000- × 1000- × 1000-μm structure demonstrating misaligned units due to block arrangement parameters.

The mixing procedure and analysis are as follows:

1. Procure a small container and wrap it in aluminum foil.
2. Measure the weight of the container and record it.
3. Now add a considerable amount of IP-S into the container (about 1–2 cm from the bottom of the container).
4. Again, measure the container with the IP-S in it and record it.
5. Determine the weight of IP-S in the container and then use that number to determine the concentration of Cu acrylate you want to add to the mixture.
 - a. Calculate by solving for x :
 - b. $x = (\text{Wanted concentration}) * (1 - \text{Wanted concentration}) * \text{Weight}_{IP-S}$
6. Add the amount of Cu acrylate to the container as calculated, using the scale to monitor the amount added.
7. Calculate the final percent concentration of the mixture after measuring the total weight.

- a. $Final\ Concentration = \frac{Weight - Weight_{IP-S}}{Weight}$
8. Use the high shear mixer to mix the mixture uniformly.
 - a. Do so three times over 1-min intervals at 6000 rpm. After 1 min, stop the mixer and remove the mixer head from the container, then repeat after 10 s.
 - b. This is to prevent the IP-S from heating up and polymerizing before even being mixed completely and used for printing.
9. Once mixed, close the container.
10. Remove the mixer head from the high shear mixer by pulling the tab on the mixing column and place the head in a beaker of IPA.
11. Place the beaker in a sonicator for 10 min.
12. Afterwards, replace the mixer head on the high shear mixer.

When trying to print using an IP-S mixture with 5.6% Cu acrylate, the Nanoscribe was unable to manually find the interface of the substrates. This prevented the 2PP tools from finding the initial resist-substrate interface needed to initiate polymerization. We were therefore unable to produce a structure with Cu acrylate within it. Further investigations will be needed to produce a useable photoresist as well as parameters for pyrolysis to keep the most metal within the carbonized structure.

4. Pyrolysis

Pyrolysis is used to carbonize the polymers on the substrate.⁷ This was used to reduce the concentration of IP-S and increase the concentration of metal additives in the structures. However, due to project and funding time constraints, the authors were unable to pyrolyze any samples printed with metal additives.

This project's pyrolysis used the IVI Corp. furnace within the ARL SEMASC. Set step sizes and ramp speeds for the various temperatures needed to uniformly pyrolyze the structures were used to program specific recipes with this furnace. The parameters used are shown in Table 1. These parameters were selected after a few test runs using nondoped IP-S photoresist.

Table 1. Parameters used in the IVI Corp. furnace to pyrolyze the microfabricated structures.

		Initial temperature (°C)	Final temperature (°C)	Process duration (min)	Ramp speed (°C/min)
1	Ramp	25	275	50	5
2	Soak	275	275	45	...
3	Ramp	275	400	38	3.33
4	Soak	400	400	60	...
5	Ramp	400	900	167	3
6	Soak	900	900	60	...
7	Ramp	900	25	60	14.6

The processes mentioned in Table 1 (i.e., Ramp and Soak) ensure that the IP-S structures evenly carbonize. Ramps are used to increase the temperature of the vacuum chamber at a certain rate per time, whereas Soak is used to maintain a temperature for a certain amount of time. The soak process allows the structure to heat evenly and slowly shrink as it carbonizes to keep its uniformity.

The pyrolysis procedure is as follows:

1. Follow the procedure for printing (listed under Basic Procedure for Printing using the Nanoscribe).
2. Locate thermal grease around the IVI Corp. furnace.
3. Apply thermal grease to the silicon base wafer and place sample on top.
4. Secure the wafer to the bottom of the vacuum chamber.
5. Close vacuum chamber and screw it shut.
6. Move to the monitor and locate the “Recipes” window.
7. Select the “IP-S 60min” recipe and open it.
8. Make sure the parameters set for the recipe are the same as above.
9. Save the recipe and then load it.
10. Select “Run” at the top of the screen.
11. The operation takes about 8 h.
 - a. Pyrolysis should start in the mornings; that way you can check on the furnace during the day to prevent the computer from freezing and keeping the furnace stuck on one temperature.
12. When the operation is finished, unscrew the lid to the vacuum chamber and remove the base silicon wafer from the chamber.
13. Remove the sample from the wafer and place the base wafer to the side.

5. Discussion: Limitations and Drawbacks

Throughout the duration of this project, there were several notable setbacks and roadblocks. A major issue that arose midway through the project was the Nanoscribe PowerScaling was reduced to about 0.7. Unfortunately, we were not

able to conduct any analyses on the effects this change caused to our prints. Ultimately, the tool issue was fixed by purchasing and installing a new laser for used for polymerization.

A more CAD-specific issue that was faced was that the entire structure was made from bottom-up before employing the Global Variables technique. In the future, a Global Variables approach should be undertaken at the onset of the project to allow facile design editing.

Additionally, the pyrolysis process also took several attempts to determine the best temperatures for the ramp and soak processes. The temperature ranges initially engaged were a bit larger with faster durations than the values provided in Table 1. Despite fewer tests, the tests only apply to IP-S and may change if larger structures ($1 \times 1 \times 1$ mm and above) were to be pyrolyzed.

6. Conclusion

Micro- and nanofabrication are crucial for defense technology, enabling the development of advanced materials and systems. These techniques create miniaturized devices, such as sensors and electronics, that enhance surveillance, communication, and navigation capabilities. Nanomaterials also improve armor, explosives, and propulsion systems. Our project aims to combine bioelectronics, micro- and nanofabrication, and capabilities development.

ARL's SEMASC uses precise tools and techniques to manufacture nano- and microscale features. The 2PP process, using the Nanoscribe Photonic Professional GT 3D printer, can fabricate structures with 220-nm resolution. This process can create complex, small structures with IP-S photoresist, which can be pyrolyzed to form conductive bioelectrodes.

However, the project faced setbacks including a reduction in the Nanoscribe PowerScaling, which was later resolved by installing a new laser. CAD design issues were also encountered, highlighting the need for a Global Variables approach from the outset. Additionally, the pyrolysis process required multiple attempts to determine optimal temperature ranges. Initial tests used larger temperature ranges and faster durations, but the values in Table 1 offer a more refined approach for IP-S pyrolysis. These findings will inform future projects, particularly when working with larger structures.

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List of Symbols, Abbreviations, and Acronyms

2PP	two-photon polymerization
3D	three-dimensional
ARL	Army Research Laboratory
CAD	computer aided design
Cu	copper
DEVCOM	U.S. Army Combat Capabilities Development Command
ECOGEB	Electrogenetically Controlled Gene Expression Bioelectrode
IPA	isopropyl alcohol
PGMEA	propylene glycol monomethyl ether acetate
SEM	scanning electron microscope
SEMASC	Specialty Electronic Materials and Sensors Cleanroom

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