Final Report

SDEC23-05

Creating DNA from Scratch for a DNA based Storage

Client/Advisor

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Hardware Team: Caden Wortman

Microfluidics Team: Anna Hackbarth, Rachel San Agustin, Astha Upadhyay

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Introduction

Problem Statement:

DNA sequencing is well-developed, but DNA synthesis is expensive and difficult to access in small quantities. This project has a wide range of potential applications in fields such as data storage, biotechnology, medicine, and genomics.

Project Scope & Purpose:

To facilitate small-scale synthesis, we will use custom-built software in tandem with integrated hardware and a modular flow device to create DNA fabrication technology. This will be used to make DNA strands for testing and research purposes.

Project Assignments & Roles:

- **Evan Walters** is one of the two leads for the GUI development for sending and receiving user input, as well as client interaction for software issues.
- **Colin Frank** is the other of the two leads for the GUI development for sending and receiving user input, as well as client interaction for software issues.
- **Caden Wortman** is the lead for hardware design and testing, as well as housing design and implementation.
- **Anna Hackbarth** is one of the three leads for the development of the Microfluidics Flow System, as well as client interaction for Flow System issues.
- **Rachel San Agustin** is the second lead for the development of the Microfluidics Flow System and helped integrate the GUI with the Microfluidics software development kit.
- **Astha Upadhyay** is the third lead for the development of the Microfluidics Flow System.

Requirements & Constraints:

Functional Requirements

- Software:
  - User-Friendly Interface
  - Intuitive front-end allowing customizable user inputs
  - Communicates with hardware and fluid control
- Image Generation:
  - The back-end processes the DNA string, creating an 'image' on the projector
  - Flip every other nucleotide string to ensure complementary strand
- Microfluidics System:
  - One nucleotide solution can flow at a time, and this is determined by the GUI which communicates to and controls a switch and air compressor.
  - Modular system – easy to expand to fit needs.

Nonfunctional Requirements

Software:
- o Graphical User Interface (GUI):
    - ▪ Recommended array size
    - ▪ Capable of conversion to a .exe file for convenient execution.
- Image Generation:
    - o Small size constraint to fit into the physical housing of the unit.

Engineering Constraints

- Standard controlled lab environments with temperatures ranging between 55° F and 85° F, level operating surface with no outdoor exposure.

Engineering Standards:

- Software and Hardware Standards
    - ○ Guidelines for Life Cycle Management – 24748-1-2018
        - ▪ This guideline is prevalent to this project because it is an inherited project that had many different design stages as explained in *Appendix II.* The Life Cycle Management Guideline outlines specific concepts, models, stages, processes, process application, and key points of view regarding the life cycle of projects.
    - ○ Test Processes – 29119-2-2021
        - ▪ The Test Processes Guideline specifically references certain test processes to be used when developing new software. It highlights lifecycle testing practices for software development.
    - ○ Systems and Software Integration – P24748-6
        - ▪ The third guideline that pertains to this project offers insights into how the integration process intersects with various life cycle processes. It gives requirements and guidance during the planning and performing of the integration of a system.

Users & Intended Uses:

Users

- Geneticists
- Data Analysts
- Pathologists
- Biologists
- Agriculture
- Bioenergy or biofuel

Uses

- Synthesized DNA is used as a form of digital data storage
- Synthetic DNA can be used for disease research
- The system can be used to simulate the naturally occurring process

Security Concerns & Risk Management:

Security Concerns

- We address security concerns by having the program run locally
- Verify that the lab is locked where DNA printing takes place, and ensure restricted access to avoid tampering

Risk Management

Risk Factors	Risk Mitigation
UV Light Exposure	• Ensure individuals receive safety training and wear UV light glasses when the projector is plugged in. • Hardware modifications, improvements, and housing attachments are made with UV projector unplugged.
Securing the Projector to the Unit Issues	• ETG manufactured a metal bracket, and we used spacers and specialty screws to mount the projector to the board.
Integrating Microfluidics Software with GUI	• Change GUI platform to Visual Studio to better communicate between the GUI and Microfluidics software.
Leakage in the connection points of the Flow Cell	• Precision gluing and cleaning ○ Glue and pieces should cure for more time in the oven, and the flow cell should undergo a second glue application and curing phase.
Clogging of the pipe in the flow system	• UV glue clogged exiting tube for fluid ○ A new flow cell must be created, or the exit tube cleared with a small pick.

Project Plan

Project Management:

The project management style we adopted for our design is an agile waterfall. Due to the technical nature of our project, we required a well-thought-out process and series of steps which allowed for the best progression of our project. We need to control the placement of the DNA base pairs which we accomplished by curing the nucleotide solution in place with UV light. We needed to create a process that allows for the flow of DNA molecules into the system to complete the DNA synthesis, which was accomplished by the microfluidics system. Due to the reliance between sub-teams, we needed a management style that would build upon previous steps, but still allow flexibility for design changes.

Task Decomposition:

Tasks and Subtasks

- Create a user-friendly interface to take custom input.
- Upgrade LCD board to UV projector.
    - Test resolution of the projector.
    - Manufacture plastic projector housing.
- Create microfluidics flow cell for DNA printing.
- Utilize software development kit to test functionality.
    - Determine basic pressure settings, valve switch code, etc.
- Integrate control of microfludics system and UV projector into GUI.

Milestones & Timeline:

Month	Software	Hardware	Flow System
August	- Summer catchup meetings	- Summer catchup meetings	- Summer catchup meetings
September	- Further develop the GUI to prepare for the integration of other units - Ensure usability and functionality	- Order UV projector - Troubleshoot and attach new hardware	- Setup microfluidics software development kit - Troubleshoot
October	- Switch to Visual Studio - Begin coding in projector control - Begin integrating Microfluidics software	- Run unit tests regarding the resolution - Create projector housing	- Learn flow cell construction process and methods development kit - Create cells - Begin testing flow system code portions
November	- Final changes to GUI - Integration Testing	- Mount projector to unit - Integration testing	- Finish creating cells - Test pressure settings - Integration testing
December	Presentation	Presentation	Presentation
Total Hours:	45-60 hours	45-60 hours	45-60 hours

Design Plan

Implementation Details:

We have three main components to this project which are: Fluid System, GUI, and Projector.
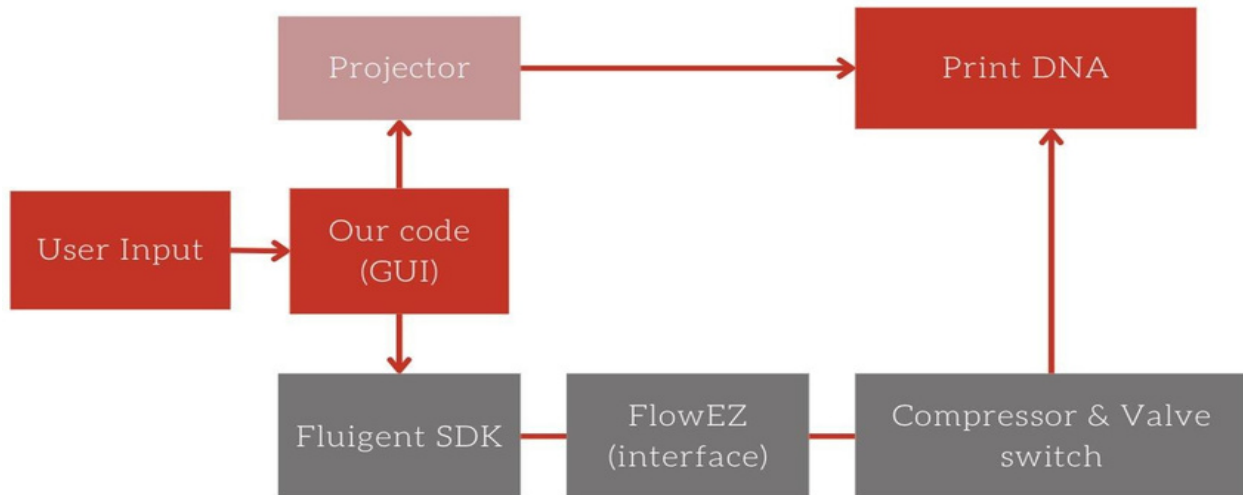

Figure 1: Whole System Breakdown

- Fluid System:
    - Software Development Kit (SDK) in C# provided by Fluigent
    - The system also has: Pressure Adjustments, Sensor Readings, Nucleotide Solution, and valve-changing mechanism
- GUI:
    - User inputs allow customization of DNA printing process
    - Target Framework: .NET Core 3.1
    - Written in C# using Visual Studio
- Projector
    - Ultra-Violet Projector from SICUBE
    - HDMI Connection

Those are all short descriptions of the individual components of the project. Integrating the Fluid Flow and GUI was most difficult because of target framework issues. Please refer to Appendix II for more details. The biggest change we made to integrate Fluid Flow and GUI was to change from a timer to Task.Delay()" to avoid threading issues.

Integrating the projector into the project plugging HDMI cord into computer running the program.

Testing Process and Results

We test all component's individually before integrating it all together and doing a whole system test. Our results from our unit and system tests are provided below.

Projector:
- Unit Tests
  - Energy Transfer and Resolution
    - Done using photolithography on AB photoresist
    - After various trials found the average time needed for energy transfer is 45 seconds
    - Applied the 1951 USAF resolution test chart to find the resolution
      - We then found we needed to add a 5mm lens extender to increase the resolution
- System Test:
  - The projector integrated with the code well and turned on at the proper intensity for the correct amount of time.

GUI:
- Form 1 and 2:
  - Tested during development, excluding external software.
  - Near completion in the project's infancy, GUI operated as a PowerShell script, emphasizing user input over logic.
- Form 3:
  - Tested in stages.
  - Initially used a timer for pulsing effect.
  - Changed to 'for' loop due to threading problems

Fluid Flow System:
- Performed basic testing functions including setting pressure, valve switching, and cycle tests.
- Pressure Tests:
  - We determined that pressure should be set between 500-750 mbar to start fluid flow.
- Flow Cell Tests:
  - Found a glue clog in the waste or wash tubing which slowed the wash cycle between nucleotides.
    - Clearing the tube with a small pick helped.
  - Leaking connections between flow cell and installed piping.
    - More curing time and a second application of glue helped.

After integrating it all together we did a unit test following Appendix I.

Demo: Unit Test

Product Comparison:

Group 5 Project
        Cost: $5,000
        Cycle Time: 3.5 min
        Control: PC(GUI)
Kilobaser one-XT (Fastest)
        Cost: $28,000
        Cycle Time: 2.5 min
        Control: Touch screen, mobile app, PC

Almost all other synthesizers on the online market require a quote for pricing and are usually used or refurbished. Overall, our project achieves the goal of synthesizing DNA at a lower cost and reasonable time compared to what is available on the market.

Resources:

Rye, Connie, et al. "14.5 DNA Replication in Eukaryotes - Biology." *OpenStax*,

https://openstax.org/books/biology/pages/14-5-dna-replication-in-eukaryotes

"1951 USAF Resolution Test Chart." *Wikipedia*, Wikimedia Foundation, 25 July 2023, https://en.wikipedia.org/wiki/1951_USAF_resolution_test_chart

Hung-Yi, Henry, and George Church. "DNA Data Storage." *Wyss Institute*, 28 May 2021,

https://wyss.harvard.edu/technology/dna-data-storage/

"Fastest DNA & RNA Synthesizer: Benchtop Sized." Kilobaser, 6 Dec. 2023,

https://kilobaser.com/dna-and-rna-synthesizer/

Carter, Sarah R, et al. *NTIBIO Benchtop DNA Report*, May 2023,

https://www.nti.org/wp-content/uploads/2023/05/NTIBIO_Benchtop-DNA-Report_FINAL.pdf

Appendix I: Operational Manuel

1.0 General / Safety

The following safety rules must be applied prior to printing DNA

- Verify all lab safety certifications have been completed
- Wear proper PPE
- Verify software has been completely downloaded GitHub Link
- Verify nucleotides and wash solution is filled and waste tube is empty

2.0 System Set Up

1. Make sure software opens in Visual Studio

2. How to add dynamic link library to run project
   2.1. Right click GUI app solution file in the solution explorer
   2.2. See Figure 2 for reference
   2.3. Go to "add" click on project reference
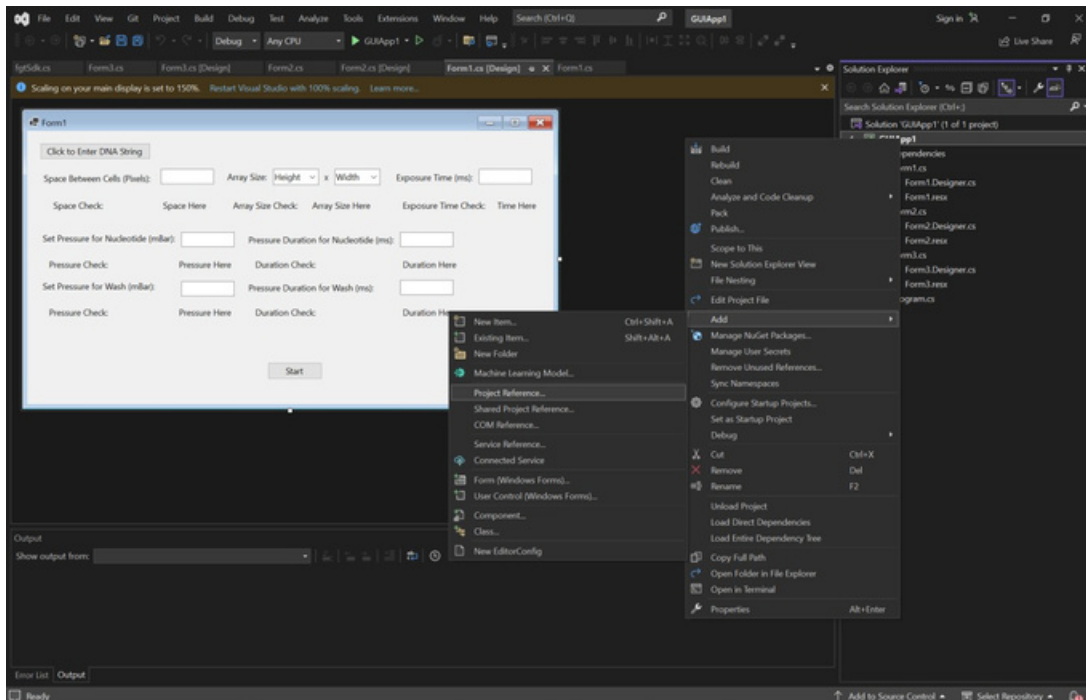
Figure 2: Referencing Project

2.4. Go to "browse" tab and click browse button

2.5. See Figure 3 for reference

2.6. Add file path to the dynamic link library
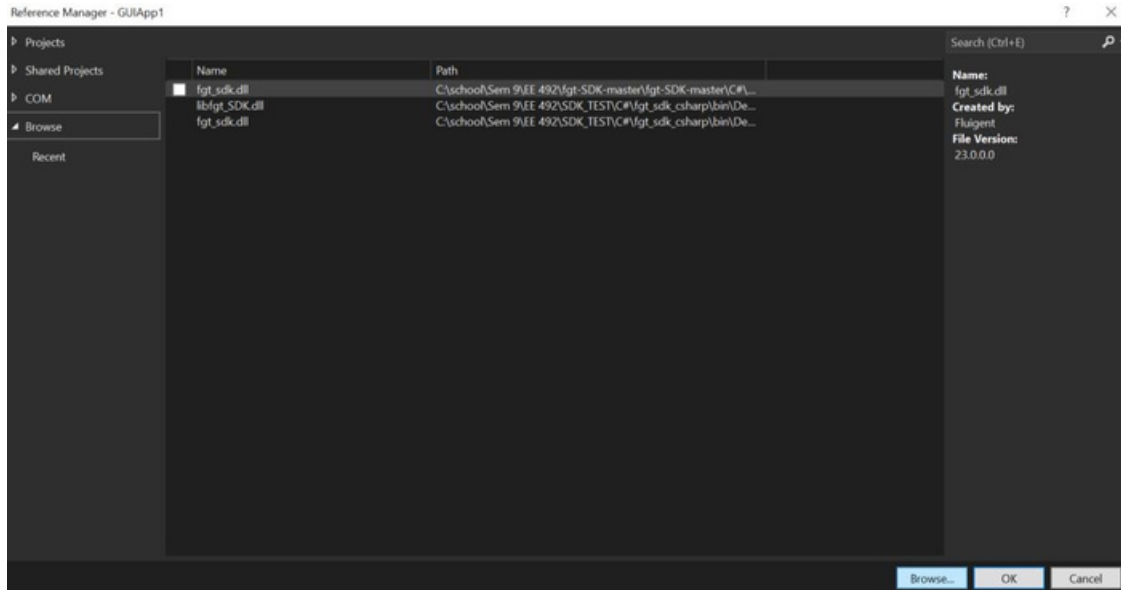
Figure 3: Adding Path File

3. Click Start button to get form 1 to open

      3.1. On form 1 click "Click To Enter DNA String"

      3.2. See highlighted part in yellow in Figure 4

Figure 4: GUI Form 1

4. In Form 2, paste the DNA strand

    4.1. Remember the recommended array size; and click save on form 2

5. Fill out all user inputs in form 1

    5.1. Click "Check/Save" button and double check user inputs before hitting start

    5.2. See highlighted part in orange in Figure 4

    5.3. Click start button when all user inputs look correct

6. Form 3 will open automatically in full screen on your computer screen

    6.1. Verify everything is correct before hitting start button to start the printing process

Appendix II: Alternate Designs

All Versions of Graphical User Interface (GUI):
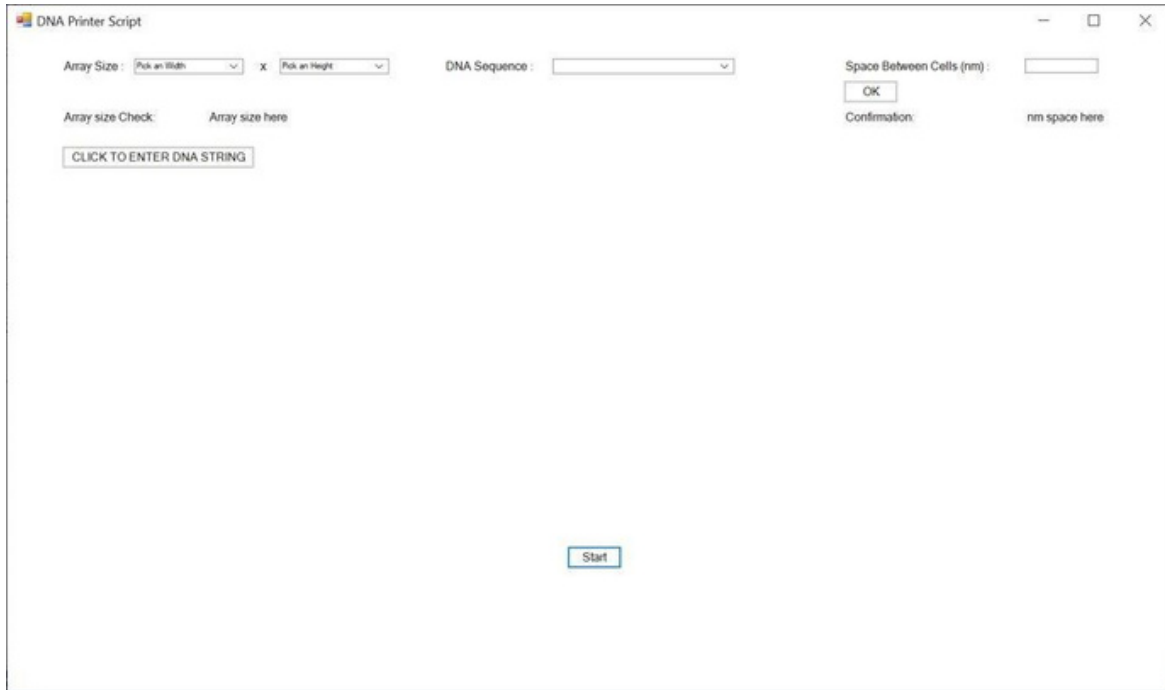
GUI 1.0

Figure 4: Powershell GUI

This version was created in PowerShell and all assets were hard coded into each form. User input allows customization of DNA printing process. The Check/Save button saves information in global variables for later use. This is a very basic version of the GUI, but it has enough functionality to interact with the projector. As the project progressed, we realized there was a lack of communication compatibility with the fluid system.
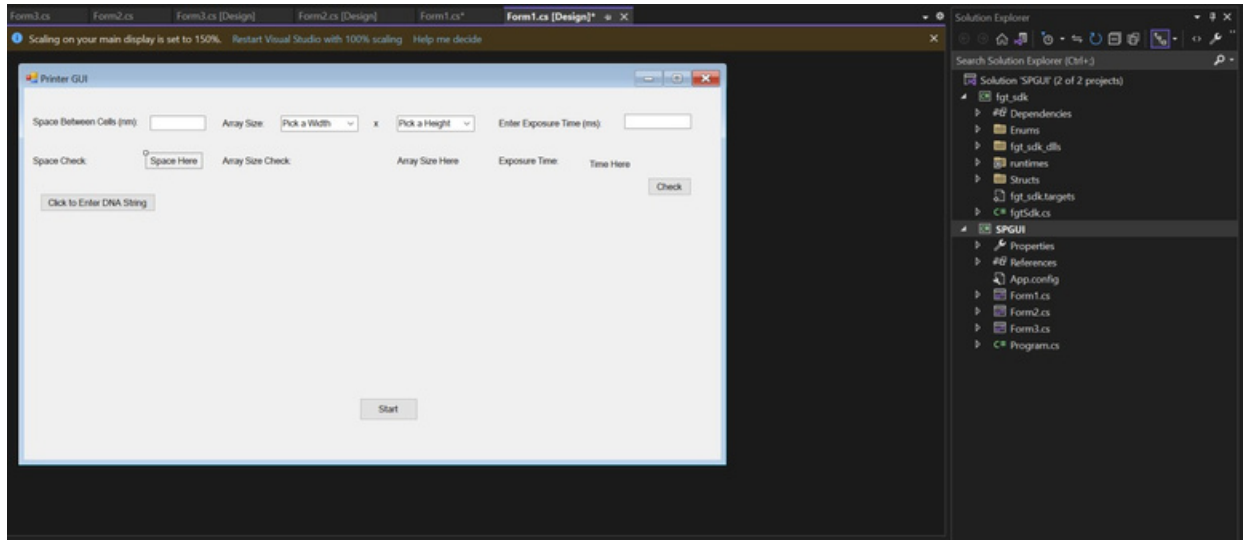
14

GUI 2.0

Figure 4: .NET Framework 4.7.2

The second version of the GUI was converted into C# and ran on Visual Studio using a target framework of .NET Framework 4.7.2. This was the newest target framework that windows forms could be developed on. After adding the dynamic link library (dll) to the project as a project reference, the software team was faced with another issue. The dll was written with target framework .NET Core 3.1, an older version. So, the team moved onto the third and current variation.
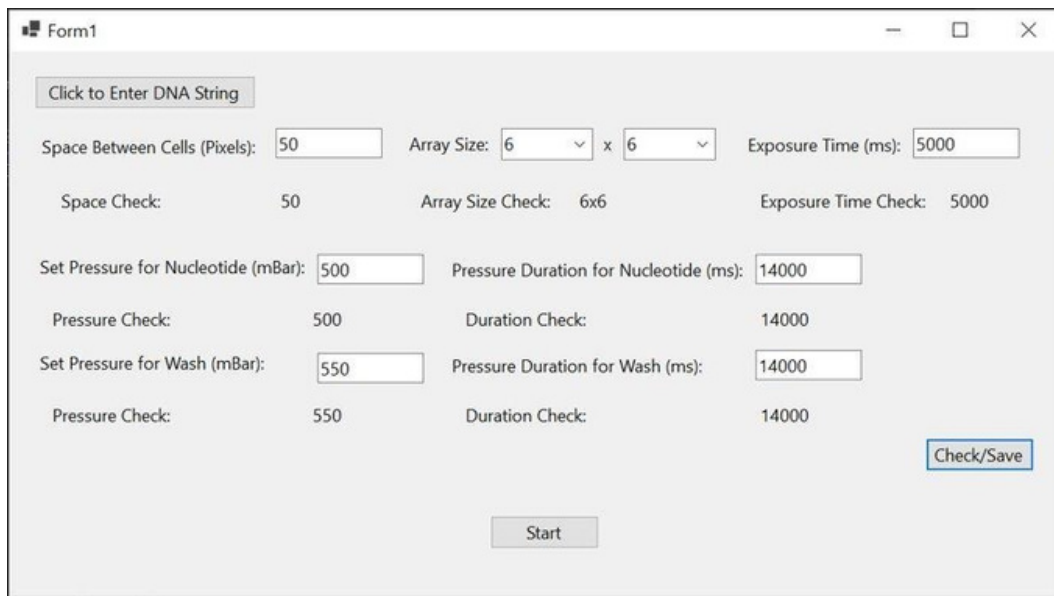
GUI 2.1

Figure 5: Current Version

Version 2.1 is still written in C#, just with the correct target framework. The group is now able to communicate with the fluid flow system. An issue dealing with the original timer arose but was solved with the use of asynchronous 'await' functions and an all-encompassing 'for' loop to facilitate any size on string.

All Versions of Hardware:

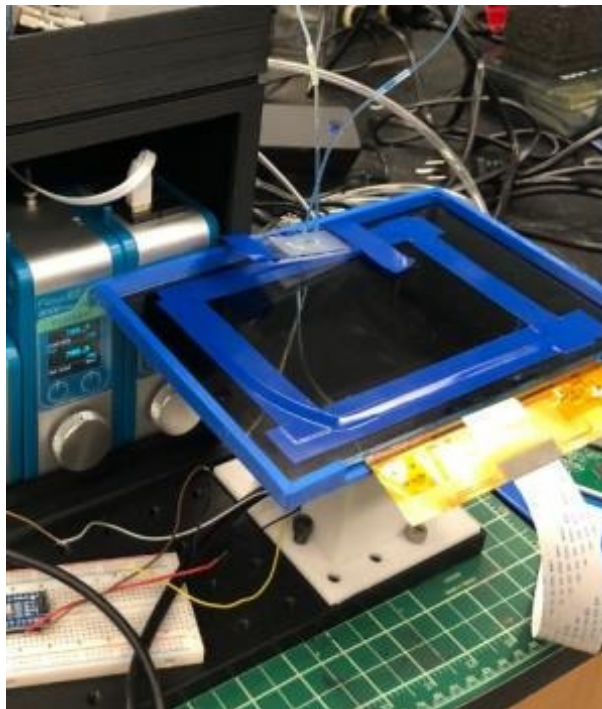**Liquid Crystal Display (LCD) Screen**

Figure 6: LCD Screen

The original design incorporated an LCD screen pressed up against the flow cell. This design, however, was highly susceptible to overheating. Also, since the LCD screen was so close to the flow cell, the excess heat would be too much for the building DNA strands and it would destroy them.

First Iteration Projector

The initial projector we decided to use in place of the LCD screen was a Texas Instruments LED projector. The blue LED was replaced by an ultra-violet LED to speed up the bonding process. However, this projector also suffered from overheating with the new LED and could not function properly for more than one minute.
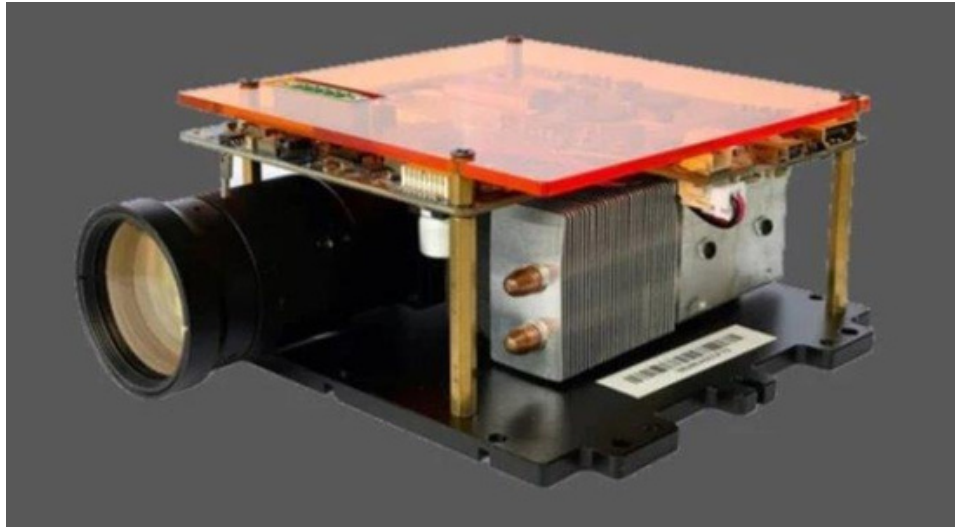
Second Iteration Projector

Figure 7: UV Projector

The second and final projector the hardware team chose was a SICUBE ultraviolet projector meant for DNA synthesis and resin 3D printing. This new projector was meant for our specific use case and had a much larger heat sync for heat dissipation. The display can be connected by an HDMI connection for ease of use with a laptop or PC containing the GUI.

Microfluidics System:

First Iteration Microfluidics (Control)

We began experimenting with OxyGEN, a user-friendly, real-time application for microfluidics control developed by the creators of our modular flow control system. While incredibly easy to use, we found that OxyGEN could not interface with our developing code, making it a poor choice as it would require the use of multiple monitors or computers to run the system. See figure to right featuring a computer with the OxyGEN application running the microfluidics system.
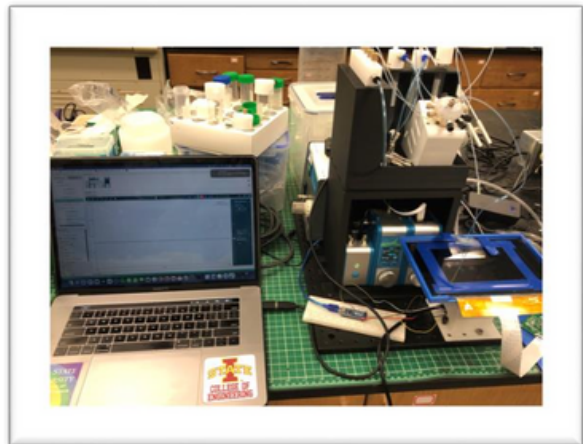
Second Iteration Microfluidics (Control)

Our last iteration saw us utilizing the software development kit in C# from the creators of our modular flow control system. This software kit was able to be integrated directly into our GUI and control the system from one computer.

Appendix III: Code

GitHub Link