EC552/BE552 - Computational Synthetic Biology For Engineers

Homework 2
Spring 2022

March 18, 2022

BioElectronic Design

On Pace: 04/15/22

Due: 04/22/22

Overview

Goal: Form an understanding of the complexity of microfluidic design problems and how computational design tools can be used to build them. In order to help build your skill-sets towards lab automation, you will be required to design a microfluidic device platform *DeltaV* that would enable the design and development of modern day gene therapeutics.

The homework is divided into multiple discrete activities, each of the activities will help you understand how to approach the problem step-by-step manner and work towards the final solution.

In order to develop the *DeltaV* platform, you will be extending Neptune, 3DuF incorporate the microfluidic component designs given in the following references[1, 2, 3] as a baseline.

> **Note:** Ensure that you do not leave this assignment for the last day, the activities are designed such that you can spread the learning a whole new field of engineering over the span of 4 weeks.

Activities:

1. **(75 Points)** Find a published gene therapy paper and write down the protocols used to develop/optimize the therapy. Your report must include the following information about the protocol:
   - Protocol Motivation (REPORT)
   - Protocol Steps (SEPARATE, REPORT)
   - Protocol Materials (REPORT)
2. **(75 Points)** Characterize the hybrid continuous-flow based microfluidic devices [1, 2, 3] and any other devices that can perform the protocol steps necessary for performing the protocol. Your report must include the following attributes about your devices:
   - Dimensions (REPORT)
   - Parameters (REPORT)
   - Valve control sequences (when applicable) (SEPARATE)
   - Fabrication techniques (REPORT)
3. **(75 Points)** Use 3DμF to create the device. Deliverables include:
   - Github Pull Requests for new components (SEPARATE)
   - SVG file (SEPARATE)

- JSON file (SEPARATE)
- 3DμF Render of the Design (REPORT)

4. **(75 Points)** Use Neptune to automatically place and route your chosen device.

   (a) Create a MINT description of the device (REPORT, MINT)

   (b) Use Neptune to place and route the MINT description. Deliverables include (SEPARATE):

   - SVG design output of Fluigi

   (c) LFR Description (Graph) (.lfr) of the Device (SEPARATE, REPORT)

   (d) MINT Netlist of the Design (.mint)

   (e) Fluid Interaction Graph (REPORT)

   (f) JSON entry for LFR Library (.json) (SEPARATE)

Note 1: Grading will be done on the deliverables so please ensure that all the required deliverables are included to ensure proper grading.

Note 2: The final design report submitted **must** in the format of a Lab on a Chip Communication Template (Overleaf LaTeX Templates).

Note 3: All reports must include a Creative Commons License that will allow us to showcase your work on the Neptune website(`https://creativecommons.org/share-your-work/`) as case studies. In case you do not want your work to be displayed or shared, please contact sanka@bu.edu.

Activity #1

In this activity you will do a literature search for a laboratory research method and outline the protocol for gene therapy

The deliverable for **Activity #1** is a report that **clearly** describes the following:

- The protocol or research method being investigated
- The importance of the protocol
- The reagents and corresponding volumes of the protocol (in a table)
- A clear description of the experimental protocol your device was designed to perform

Activity #2

In this activity you will do a literature search for a set of continuous flow microfluidic device that can either be used to implement the protocols used in the previous activity. The device **must** be based on continuous flow (not electro-wetting/digital-droplet, etc.)). Your device can have valves that are pneumatically actuated. The following journals are a great place to begin your search for an interesting device. This by no means an exhaustive list:

- Lab on a Chip
- Microfluidics and Nanofluidics
- Biomedical Microdevices

In order to make the best decision regarding which device to analyze for this assignment, please read the entire homework assignment before selecting a device. This is to ensure you understand what you will be doing with this device. Points will be awarded based on the quality of the deliverables as well as how interesting and complex the device is (an extremely simple device that does not lend itself well to designing with tools will have a hard time earning top marks in this assignment).

The deliverable for Activity #2 is a report that **clearly** describes the following:

- The device's physical dimensions
- What are some of the device's parameters (channel width, number of inputs, number of valves, etc.)? How does the function of the device change as these parameters change?
- How does this device scale? Can you tile the device to make larger devices? Cascade the device and do intermediate operations?
- A temporal description of the valve control sequence(when applicable).
  - Hint: You should clearly link the physical architecture of the device to the experimental protocol that the device was designed to perform. All intermediate steps performed on the microfluidic chip should be clearly documented here.
- A description of the fabrication technique used to create the device. Popular techniques are soft lithography, CNC milling, wet etching, etc.

Activity #3

3DμF is a user-friendly CAD tool for placing microfluidic components on a grid and exporting the design into a few useful file formats. Your job in Activity #3 is to use 3DμF to design the device designed in Activity #2 and export the device in three file formats: JSON (to send to microfluidic control software), SVG (for use with a CNC mill), and STL (for 3D printing).

It is very likely that 3DμF [4] will not have all of the components you need to properly design your device. Your job will be to clearly describe features that 3DμF cannot draw and create new components for each one that 3DμF does not support. You can find the guide to creating new components in Chapter 6 of the Supplementary Information of 3DμF [4]. You will be required to create the missing components from [2, 1, 3] to ensure that the platform library you create is useful beyond a single device.

Deliverables for Activity #3:

- Include the Github Pull Requests references for new components
- SVG file
- JSON file
- Pull request implementing the new component to 3DμF, including primitive definitions for each feature.

CAD Tutorials

- 3DμF Tutorial - `https://www.youtube.com/watch?v=YOrnnZjma28`
- Adding a new component onto 3DuF Tutorial - `https://www.youtube.com/watch?v=JmOGMJDbIi8`

Activity #4

Neptune is a specify, design, and build tool for the development of continuous flow microfluidics. With Neptune, users can specify microfluidic devices through a high level description of liquid flow relations (LFR). The application will automatically generate the physical design of the microfluidic device that can opened in 3DμF for making edits or generating the designs for fabrication. Neptune is currently available at `http://fluigicad.org`.

Neptune comes with the Fluigi tool chain which can take in two kinds of inputs: a) Liquid Flow Relations (LFR) file b) Microfluidic Netlist (MINT) file [5]. In this activity you will create both an LFR file and MINT file that sufficiently describes the microfluidic device.

In order to simplify the design, you can use Visual Studio Code and install the plugins for MINT and LFR.

With LFR:

1. Create a description of the of the protocol/device using LFR, Liquid Flow Relations
2. Include comments into the LFR description file.
3. Generate FIG and MINT file from LFR description

With MINT:

1. Create a description of the of the device using MINT, a microfluidic hardware description language.
2. Include comments into the MINT description file.
3. Place and route the device using Neptune.

Note: Since Neptune is actively under development. You might face some problems in using the tool. If you face any problems please make sure you create an Issue on Github, with screenshots and error messages so that they can be addressed quickly.

Deliverables for Activity #3:

1. LFR description of the protocol/device named eg. 'device.lfr'
2. Platform Library JSON. 'deltav.json'
3. Rendered FIG graph of the LFR design in the report (PDF).
4. MINT Description of the microfluidic Device named eg. 'device.mint'.

5. Clearly document the features that could not be implemented using MINT (REPORT) showing images and the alternative components that were used in place of the original feature.
6. ~~Brief note on the changes incorporated in the PAR/DRC parameters for the device in the report.~~
7. Brief note the Physical Design Rules necessary for devices. These can be factors that prevent the device from being used in practice (from factor) or prevent it from functioning.
8. ~~EPS design file of the generated design.~~
9. Log generated by Neptune.
10. Fabricated Device

Dealing with Fluigi/MINT Error

Since Fluigi is a research tool in active development, there might be some constructs or scenarios where the application will not work as expected or might not accurately report the errors in the MINT file.

The binary version of Fluigi that will be distributed will not have some of the actively developed functionality, namely the verilog device generation and the hierarchical design parsing (i.e. the 'IMPORT device' option).

In many cases the Place and Route (PAR) might fail because it does not find a good solution or finds a solution that would violate the Design Rule Check (DRC). In these event's the report should include all the failure messages and the snapshots generated by Fluigi to avail the bonus points and to demonstrate the limitations of the active research tool.

In the scenarios where Neptune crashes or fails, Github issues should created for each of the scenarios on the homework/Fluigi Github repository. This way the questions can be cleared quickly and the binaries can be patched to fix the errors. In addition to that if faced with any other difficulty please contact sanka@bu.edu requesting assistance/office hours.

LFR Tutorial

LFR is a microfluidic hardware description language used for describing microfluidic circuits and devices. Just like any other Hardware Description Language like Verilog or VHDL, LFR aims to help microfluidic designers leverage the use of CAD tools by allowing them to describe complex architectures in a human and machine readable format. By using LFR descriptions of the hardware. The designers can easily design microfluidic devices without the need of learning how to use mechanical CAD tools and learning how fluid dynamics work. A guide for learning the LFR language is available at `https://github.com/CIDARLAB/LFR-TestCases/wiki`. In order for an LFR design to be synthesized correctly, the user needs to create a 'platform library' that consists of component options, mappings and algorithms that help generate the designs correctly.

Note - Detailed LFR tutorial available at https://www.youtube.com/watch?v=cM1Yrbh9Bk0

MINT Tutorial

MINT is a netlist description format used for standardizing how we represent the design in detail. Unlike LFR designs, MINT designs are a 1:1 representation of the design that is either manually created by the user or automatically generated from tool like LFR. By using MINT descriptions of the hardware. The designers can easily share and tweak the circuit and component parameters and speed up the entire experimental process.

An example of MINT Syntax can be seen fig. 1

```
DEVICE test01

LAYER FLOW
PORT p1, p2, p3 r=500;
NODE n1;
H LONG CELL TRAP ct1 numChambers=10 chamberWidth=500
chamberLength=500 chamberSpacing=100 channelWidth=500;

CHANNEL c1 from p1 3 to n1 1 w=500;
CHANNEL c2 from p2 1 to n1 3 w=500;
CHANNEL c3 from n1 2 to ct1 1 w=500;
CHANNEL c4 from ct1 2 to p3 4 w=500;
END LAYER

LAYER CONTROL
PORT cp1, cp2 r=500;
VALVE v1 on c1 w=1000 l=500;
VALVE v2 on c2 w=1000 l=500;
CHANNEL c5 from cp1 2 to v1 4 w=500;
CHANNEL c6 from cp2 2 to v2 4 w=500;

END LAYER
```

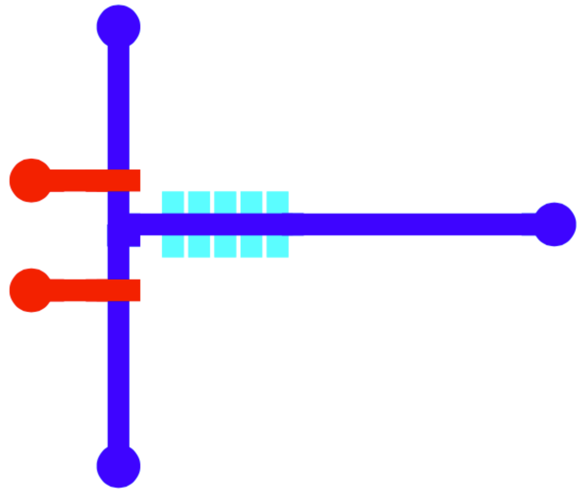

Figure 1: Netlist example. The netlist describes a simple device with three ports and a cell trap on the flow layer and two valves and ports on the control layer. The name of the device is given in the first line. The features and connections on the flow layer are described first, followed by those on the control layer. The design described in the netlist is shown on the right.

MINT FAQ

Q. How can I find the MINT documentation?

A. MINT's references can be found at MINT Github Wiki.

Q. How do I create and structure a MINT file and are there any examples?

A. The basic rules and example mint files can be seen at Netlist Rules and Examples

Q. What are the various primitives and modules available in MINT?

A. The current version of MINT compiler supports all the components on 3DμF.

Neptune Tutorial

Neptune is a specify design and build tool for Microfluidics. At its core, it contains a microfluidic place and route tool used for laying out the microfluidic components described in a MINT file and properly connecting the components according to the MINT description.

Neptune FAQ

Q. How do I get the Neptune binary?

A. Neptune can be found at Neptune Website Neptune Github Repo

Q. How do I run the MINT/LFR compilers binary on my computer?

A. The python code for pyLFR and pyFluigi. These packages when installed will provide the user with a command line interface that the user can use on their own machines. However, we only support Linux based installations.

References

[1] Fatemeh Ahmadi, Kenza Samlali, Philippe Q. N. Vo, and Steve C. C. Shih. An integrated droplet-digital microfluidic system for on-demand droplet creation, mixing, incubation, and sorting. *Lab on a Chip*, 19(3):524–535, January 2019. Publisher: The Royal Society of Chemistry.

[2] Kenza Samlali, Fatemeh Ahmadi, Angela B. V. Quach, Guy Soffer, and Steve C. C. Shih. One Cell, One Drop, One Click: Hybrid Microfluidics for Mammalian Single Cell Isolation. *Small*, 16(34):2002400, 2020. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/smll.202002400.

[3] Kenza Samlali, Fatemeh Ahmadi, Angela B. V. Quach, Guy Soffer, and Steve C. C. Shih. Single Cell Isolation: One Cell, One Drop, One Click: Hybrid Microfluidics for Mammalian Single Cell Isolation (Small 34/2020). *Small*, 16(34):2070190, 2020. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/smll.202070190.

[4] Radhakrishna Sanka, Joshua Lippai, Dinithi Samarasekera, Sarah Nemsick, and Douglas Densmore. 3DF - Interactive Design Environment for Continuous Flow Microfluidic Devices. *Scientific Reports*, 9(1), December 2019.

[5] Radhakrishna Sanka, Brian Crites, Jeffrey McDaniel, Philip Brisk, and Douglas Densmore. Specification, Integration, and Benchmarking of Continuous Flow Microfluidic Devices: Invited Paper. In *2019 IEEE/ACM International Conference on Computer-Aided Design (ICCAD)*, pages 1–8, November 2019. ISSN: 1933-7760.