Engineering Requirements for the Droplet PCR Centrifuge

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1 Introduction

1.1 Overview

This document provides a description of the centrifuge system including it's purpose, required features, required specs and required constraints.

1.2 Scope of this document

The device outlined in this document is intended as an experimental prototype and as such, the purpose of this project is solely to demonstrate the viability of the device. This means demonstrating that the device is able to reliably perform it's function as outlined in the Functions section of this document. Integration and optimization are not within the scope of this project.

2 General Description

2.1 Functions

The device is intended to carry out two major functions:

1. The first function is to generate a water in oil emulsion. Each droplet inside this emulsion will serve as a micro-reactor for performing an isolated PCR reaction. With thousands of droplets inside a sample, it will be possible to perform thousands of PCR reactions in parallel.

2. The second function is to make the sample tube available for automatic mechanical extraction. Once the emulsion is generated, the sample needs to be passed off to a thermal cycler for performing the PCR reaction. The tube needs to "pop out" in such a way that a mechanical sample transport system can grab the tube and move it into the thermal cycler. The sample transport system itself is beyond the scope of this project, but the centrifuge should have a mechanism to make the tube available to the sample transport system.

2.2 Working Principle

The working principle for generating the emulsion is as follows:

1. Fill a sample tube $\tilde{2}0\%$ of the way with oil.

2. Insert a MiCA plate into the sample tube. This MiCA insertion should include a gasket which forms a water-tight seal with the inside walls of the sample tube. The goal of the insertion is to force any solution injected in the next step to pass through the holes of the MiCA plate.

3. Fill the top of the sample tube (above the MiCA plate) with the solution containing genetic material.

4. Seal the sample tube and place it inside the swing-bucket centrifuge.

5. Centrifugate the sample tube at a high-enough RCF that the solution containing genetic material is forced through the holes of the MiCA plate. Done correctly, this will cause the solution to break off into droplets when exiting the MiCA channels. These droplets will then smash into the oil at the bottom of the tube, embedding themselves in the oil.

3 Requirements

3.1 General Requirements

- **RCF:** According to the latest study by Liao *et al.*, the minimum relative centrifugal force (RCF) required for generating the optimal emulsion using their MiCA plates is **15,000 g** [1].
- **Constant RPM:** The centrifuge should be able to monitor it's own RPM and maintain a constant RPM.
- **MiCA Plates:** These need to be manufactured and then integrated into an "insert" that can be pushed inside a sample tube. The insert should use a gasket to seal the interface between the circumference of the MiCA plate and the inside of the sample tube wall. We'll send you a paper that details the process.
- **Sample Tubes:** Everything should be designed to work with the sample tubes found at this link - (Fill in later)
- **Sample Capacity:** The centrifuge should be able to centrifugate 8 sample tubes simultaneously.
- **Safety:** The centrifuge should be encased in a shrapnel proof box during operation. The device should cut power to the centrifuge motor if the lid is open.
- **Sample Ejection:** The device should be able to eject all the sample tubes so that they can be grabbed by a "sample transport" system located outside the shrapnel proof box. I don't have any idea what the best way to do this is. Hopefully you guys have some ideas.
- **Device Footprint:** Just keep in mind that the centrifuge is going to be integrated into a larger point-of-care (POC) device. The entire POC device needs to fit comfortably on a lab bench, and the centrifuge needs to fit inside the POC device.
- **Max Power Usage:** $\leq$ 600W
- **Parts/Materials Cost:** $\leq$ \$15,000 CAD
- **Temperature Control:** None.

3.2 Functional Requirements

- The MiCA plate insertions must fit properly inside the sample tubes (we will provide a tube model number). The insertions must form a water-tight seal with the inner wall of the sample tube. The MiCA plate must be of sufficient quality that it is capable of generating emulsions that will work with droplet PCR.
- The centrifuge must be able to centrifugate the samples at $\geq 15,000$ RCF for ≥ 7 minutes.

- The device should make the sample tubes available for automated mechanical extraction once centrifugation is completed.
- The device must create a water in oil emulsion inside each sample tube. This water in oil emulsion must be of sufficient quality to perform an accurate PCR reaction + reading on the samples.

3.3 Hardware Interface

The device should have a mechanical interface which ejects the sample tubes such that an external sample transport system can easily grab the tubes.

3.4 Software Interface

The device should have an API that allows us to configure and control the device programatically. It doesn't need to be anything fancy. Just a CLI that let's us configure and control the device. I'm doing all our other sub-systems in Python so it would be beneficial if you guys could use Python too. If you have other ideas just let me know and we'll talk about it. My goal is to produce a distribution package for each sub-system and provide a simple CLI to each package. This keeps our sub-systems decoupled and lets us easily combine them in arbitrary ways for other projects. It also makes CI/CD easier whenever I finally set that up. This is all just made cleaner/easier if we stay within the Python ecosystem. And I feel Python is a good choice for a prototyping language anyway :D

3.5 Similar Devices

(https://www.thermofisher.com/order/catalog/product/75007210) this device is similar in specs to what we need. However, nothing we've found off the shelf has an automatic sample ejection feature or an easy way to interface physically and programatically. Ours doesn't need to be as fancy or refined as the one linked. We only care that it works reliably and safely.

4 References

References

[1] P. Liao, M. Jiang, Z. Chen, F. Zhang, Y. Sun, J. Nie, M. Du, J. Wang, P. Fei, and Y. Huang, "Three-dimensional digital pcr through light-sheet imaging of optically cleared emulsion," *Proceedings of the National Academy of Sciences*, vol. 117, no. 41, pp. 25 628–25 633, 2020.