Alternative Designs Report

Thermoacoustic Cell Sorter

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1 Scope

This document covers different design options being considered for the thermoacoustic cell sorter. It is assumed that the audience is familiar with the proposal for this project.

2 Introduction

The thermoacoustic cell sorter is basically composed of three separate parts: the flow control module to ensure constant movement of cells through the system; the electronic decision making module to determine the appropriate bin for each cell; and the gate mechanism to ensure that cells reach their intended bin. Each of these parts can be interchanged without greatly affecting the other parts. So rather than presenting different designs, we would like to discuss the different components of the design and the different options we are considering for each part. Our goal is to maximize the accuracy of our prototype while minimizing wasteful expenses.

3 Flow Control

Before any information can be gathered about the cells, and before any sorting takes place, the cell stock needs to be contained in a proper fashion. To accomplish this, the unsorted cell stock will be placed into an IV bag. The IV bags will be 100mL, 250mL, or 500mL depending on what our client wants. The bag will then be attached to the capillary tube in order to allow flow through the device. There are three different approaches that can be used when considering the attachment of the saline bag to the capillary tubes. The different methods affect the cellular flow for the TACS device in different ways.

3.1 Flow from the System to the Holding Bins

3.1.1 Rubber Stopper

The first approach to be taken is simply to use a rubber stopper device that will allow for the insertion of the capillary tube directly into the saline bag. The rubber device will be tapered outward from the insertion location of the saline bag and have an opening for the .08mm diameter capillary tube. This approach will be the most cost efficient method but may create more problems in the future. This does not directly provide us with a flow rate before we start testing, nor does it provide us with a way of regulating the flow rate once the device is complete. If this rate is too slow, or too fast, we will need to purchase and incorporate a flow regulating device to manage the problem later in the design process, this situation is not ideal.

3.1.2 IV Flow Regulator

Another approach allows us to regulate the flow directly by using an IV flow regulator. These devices are used in hospitals to regulate the delivery of medicines that are contained in saline bags. An IV flow regulator can directly regulate the cellular flow anywhere from 0 to 250mL per hour. Such devices use a dial to set the rate. Knowing the
flow rate of the system is crucial for the success of the device. The drawback of this apparatus is that IV flow regulators potentially require a physician’s license to purchase. The cost is not yet known for one of these devices but a response is imminent.

### 3.1.3 Hydrodynamic Focusing

The third and final approach would be to use a hydrodynamic focusing device. Such devices use a sheath or saline solution that enters and mixes around the sample solution at a faster velocity.

![Figure 1 – Basic Hydrodynamic Focusing Device](image)

(Dean, 2007)

The faster moving liquid applies a pressure to the cellular stock in accordance with Bernoulli’s principle. The disadvantage to this device is that we would have to make one and it can not simply be purchased. All of these devices provide specific advantages and disadvantages that may help or hinder the progression of the TACS. The final destination for the sorted groups of cells will be a saline bag just like the one at the start of the system. The volumes will all be the same so one bin will be able to accommodate the complete cell solution.

### 4 Electronic Cell Differentiation Method

The method by which cells are identified as belonging to one group or another consists of a laser diode to excite and heat the cells, PVDF films of different thicknesses to detect the resulting ultrasound wave, and some system to interpret the voltage response from the PVDF membranes and then send a signal to the correct gate to open or close. In the following sections I will discuss the different options for each part.

#### 4.1 Laser Diode

The choice of the laser diode is largely based on the type of sorting which we would like to do. In different versions of the device, the laser diode is likely to change. In order to maximize the ability of the system to differentiate between cell types, we need to
maximize the differences between the voltage responses given by the PVDF films, which means we need to maximize the differences between the longitudinal wave given off by each cell type. The only way to do this is by selecting a laser diode with a wavelength which will greatly excite the molecules in one cell type, but not in the other cell type.

To illustrate this point I’d like to look at red blood cells, as they are commonly the subject of photoacoustic imaging. Consider the case of oxygenated blood and deoxygenated blood. Oxygenated blood appears red, which means that it reflects red light, whereas deoxygenated blood absorbs red light. If a red laser diode were used in this case, the molecules in deoxygenated blood would absorb more energy, whereas the molecules in oxygenated blood would reflect the majority of the incoming light. Therefore, more thermal expansion would occur in the blood cell devoid of oxygen, causing the longitudinal wave to have greater amplitude, and the resulting voltage response of the ultrasound sensors to be greater than those resulting from the oxygenated blood cell.

![Figure 2 - Absorption Spectra of Oxygenated (Red) and Deoxygenated (Blue) Hemoglobin](image)

(Prahl, 1999)

As shown in Figure 2 (Prahl, 1999), oxygenated hemoglobin absorbs much less energy from light with a wavelength between 600-700nm.

In our first test, we would like to differentiate between living and dead cells. A common method of doing so would be to expose the cells to trypan blue dye, which traverses a dead cell’s membrane but not a living cell’s. Trypan blue dye has an absorption peak at 580nm (Wollensak, 2004), which is yellow light. The closer the laser diode’s wavelength is to this absorption peak, the greater the thermal expansion of the dead cells would be. Unfortunately, laser diodes are only available at discrete wavelengths, and none of the currently available laser diodes are yellow. The closest available wavelengths of laser diodes would be 532nm (green) or 635nm (red). 650nm laser diodes are the most common laser diodes, and the least expensive.

### 4.1.1 The easy way out

The 650nm laser diode is the least expensive and easiest to acquire laser diode. A potential drawback of this option is that it may not cause a noticeable difference in thermal expansion between cell types.
4.1.2 A single-diode approach

Both the green (532nm) and red (635nm) diodes mentioned before, are about 50nm off-center from the absorption peak of trypan blue. So both diodes would likely produce the same devices of the same accuracy. These devices would be more accurate than a device which uses a 650nm diode, but would cost a bit more.

4.1.3 A multiple-diode approach

There is no reason why we could not place several diodes one after another on the capillary tube and measure the thermal expansion of the cells in response to different frequencies of light. Naturally this would be most expensive option. This would also slow down the decision making software as it would increase the amount of input which would need to be interpreted and evaluated. This would also likely be the most accurate option.

4.2 PVDF Films

The PVDF films are relatively expensive. The thinner the film is, the more expensive it is. The commonly found thicknesses for PVDF films are 110micron, 52micron, 28micron, and 9micron. 9micron film is almost as expensive per square cm as the other three thicknesses combined. The frequency range to which each film responds is related to the thickness of the film by the following equation, \( F_{\text{max}} = \frac{v}{2t} \) (Piezo, 2006).

Where \( v \) is the speed of sound in the film, \( t \) is the thickness and \( F_{\text{max}} \) is the frequency at which the film resonates.

Our options for this component range from using a single sensor, to using one of each sensor.

4.2.1 The easy way out

Using a single sensor made of the 110micron film would naturally be the least expensive approach, but also likely the least effective.

4.2.2 The most accurate method

Using one sensor of each thickness would provide the most information about the cells, and definitely yield a better data set for differentiating between cell types, but also cost the most. This would also increase the amount of computing time required to evaluate the signals.

4.2.3 The Compromise

Once we’ve tested each PVDF sensor, it will be easier to tell which signals actually contribute to the overall accuracy of the device, and which are recording the most noise. If we find that one of the sensors provides a signal which clearly differentiates between the two cell types then we could afford to use only one sensor and save money on manufacturing, and computing time.
4.3 Decision Making Module

The decision making module has the following requirements:

- Read the voltage signal from the PVDF films
- Determine the type of cell
- Output a voltage to open and close voltage actuated gates

Both design options will include a certain amount of circuitry in order to ramp up or ramp down the voltage responses of the PVDF films, and filter out environmental noise.

4.3.1 Circuitry Based Option

A circuitry-based design of the decision making module would likely cut the amount of money spent on parts, but it would not allow the use of complex signal analysis. This method would also complicate debugging, as the number of components could become very large.

4.3.2 Software Based Option

A software-based approach to solving our problem would be more expensive; it would necessitate the purchase of a data acquisition device, as well as compiling a LabVIEW or Matlab program. In our case, these are already at our disposal, but in industry, this would increase the cost of materials. This approach allows us to use complicated functions such as Fourier transforms, wavelet transforms, etc. which could provide huge increases in the accuracy of the device. Debugging the programs will be considerably simpler than debugging a complicated circuit board. So this method would be less time consuming, and yield a more accurate device.

5 Gate Mechanism

There exists a need to separate the particles into the appropriate bins. A gate will need to be designed to regulate the flow into appropriate bins. Since the overall apparatus is small, a gate that can switch between open and closed with electrical stimulation would be ideal.

5.1 Actuating Zeolite Material

The first design for the gate involves the use of a nano-porous Zeolite material. This material will be impregnated with copper. Upon an applied electric field, the material will absorb ions into the nano-pores and increase the volume of the material. This expansion will cause the material to block the flow to a certain collection bin, thus adequately separating the particles.
There are certain drawbacks of this design. This material has not been used as a
gate material previously and the performance in such an application is unknown. In
addition, the preparation methods for the zeolite material could prove costly and
troublesome.

5.2 Magnetic Gate

An alternative design for a flow control mechanism involves the use of a magnetic
material. At the correct moment, an electric field will be applied and the material will
acquire a magnetic moment. It will be directed with an opposing permanent magnet to a
particular edge of the capillary tubing. It will thus block the flow in one direction. This
method could either stop the flow in one capillary or direct the flow between two
capillaries. This then allows for particle sorting. The drawback of this method is that
securing the magnetic material in place may be challenging.

5.3 Thermally Expanding Gate

A third method is to use a shape memory material such as nitinol as the gate or a
thermally expanding material. An electric current would be applied to supply heat and the
material would expand to block the flow. It would then return to its original shape upon
cooling. The drawback of this method is that it may take time for the material to heat and
cool. This does not allow for fast actuation of the gate and thus limits the amount of
particles that can be sorted per unit time.
6 Appendix

Figure 4. An autodesk drawing of a capillary tube.

Figure 5. An autodesk drawing of a saline bag with hole at the bottom for the insertion of a rubber stopper.
7 Works Cited


Nolla, Hector. Berkley Flow Cytometry. University of California, Berkeley. <biology.berkeley.edu/crl/flow_images/fig1.gif>

