Circulating Tumor Cell (CTC) Detection with Close-packed Bead Immunoassay

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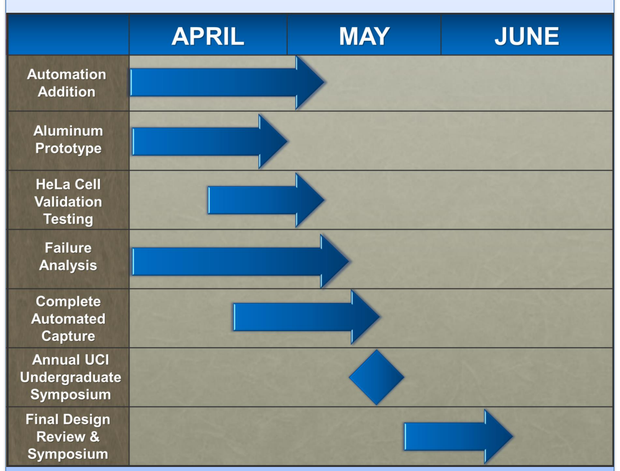
Project Goal

Our goal is to create a fast, cost-efficient system for **cancer detection of circulating tumor cells (CTCs).** A CTC detection system isolates tumor cells from blood and scans for CTC presence. Other CTC detection systems, while effective, are **expensive to produce and utilize** for point-of-care diagnostics. They use abrasive capture and separation methods like centrifuging that often damage cells in the process. Also, these devices are costly for laboratories to purchase. We will focus on **low cost and flexible sample detection** as the foundation of our design.

Project Overview

A low cost, real-time diagnostic applicable for point-of-care operations that will capture circulating tumor cells (CTCs) using **microfabricated immunomagnetic microbeads in close-packed formation**. Blood taken from a patient will be loaded into a funnel containing beads concentrated at the tip. Individual cells passing through interstitial spaces will provide full contact with the surface of the microbeads, allowing for a **high accuracy antibody-based detection** of CTCs. Magnetic alignment will provide simple microscopy targets for further analysis.

Spring 2015 Timeline



Background

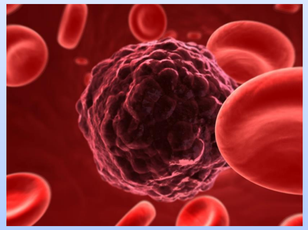


Figure 1: Graphic representation of a single tumor cell among several erythrocytes (red blood cells).

- The **second highest cause of death** in the world is cancer. An effective and cheap diagnosis test can potentially save millions of lives.
- During metastasis, there are only **1 to 10 circulating tumor cells for every 1 mL of blood** (approximately one billion red blood cells). Early detection is currently a challenge for researchers.
- **Circulating Tumor Cells (CTCs)** can be found traveling through the circulatory system during metastasis buildup. Detection and monitoring of CTCs is helpful in establishing patient-specific treatment plans and assists physicians in monitoring metastatic patients. CTCs can be characterized from normal hematopoietic cellular constituents. However, low specificity and sensitivity for CTCs hinders further clinical application.
- A **functionalized magnetic bead system** to detect CTCs is proposed in this project as a low cost, minimally invasive alternative to current diagnostic techniques such as biopsies and endoscopies.

Product Design

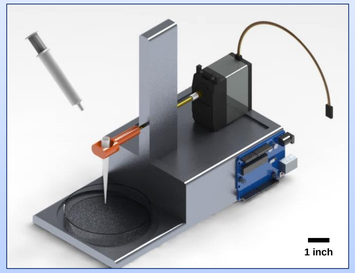


Figure 2: Solidworks CAD model of proposed system. The base is planned to be machined out of aluminum and it will feature both 3D printed parts and stock materials.

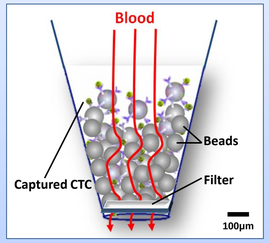


Figure 3: Immunomagnetic beads 100µm in diameter are tightly packed at the tip of a funnel. A filter near the tip will allow fluid flow while preventing beads from escaping the tip.

System Operation Guide

Step 1: Place filter inside bottom of pipette tip
Step 2: Cover filter with Immunomagnetic Microbeads
Step 3: Attach tip to syringe loaded with fluid sample
Step 4: Apply pressure from syringe using linear motor to push fluid through beads and filter
Step 5: Switch petri dish filled with fluid sample for a clean one
Step 6: Activate automation to eject tip and dump microbeads into petri dish
Step 7: Backwash pipette tip to ensure all microbeads have been removed
Step 8: Wash the microbeads to remove excess fluid
Step 9: Align microbeads spatially using magnets for easy analysis

Design Validation

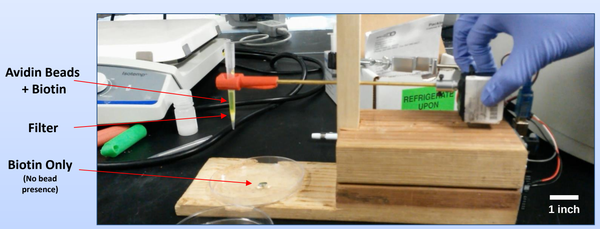


Figure 4: Validation of our system by use of Avidin-Biotin conjugation to model CTC capture. We tested filter separation of Avidin-coated polystyrene beads and fluorescent biotin. As fluid is pushed through the filter, the beads are left behind while all biotin particles not captured by the beads are collected in the petri dish below. The full automation system includes an Arduino board, servomotor, pipette tip holder, and brass rod.

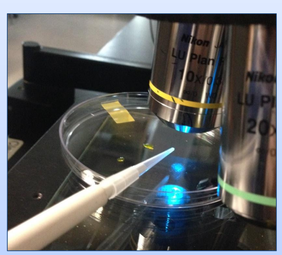


Figure 5: Validation of Avidin-Biotin conjugate fluorescence using a Nikon Eclipse fluorescence microscope. The small yellow droplet contains the filtered Avidin-coated beads.

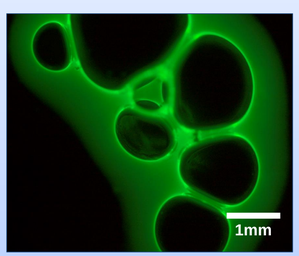


Figure 6: Images showing the fluorescence of Biotin (5-fluorescein) conjugate using a 4x objective lens. An excitation wavelength of 490nm is observed in the green spectrum of visible light.

The Team

From left to right: Joshua Yu, Luis Rodriguez, Zach Gyugyi, Aimee San Jose, and Jamison Jew

Team Roles

Lawrence Kulinsky[2]	Project Mentor	Faculty Advisor Design Review
Zach Gyugyi[1]	Executive Team Leader	Spokesman Automation Procedure
Joshua Yu[1]	Administrative Team Leader	Meeting Coordination Manufacturing Process
Luis Rodriguez[1]	Fabrication Expert	Cost Analysis Materials Selection
Aimee San Jose[1]	Biotin/Avidin Researcher	Validation Testing Clinical Applications
Jamison Jew[1]	Micro/Nano Specialist	Failure Mode Analysis Prototype Assembly

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What's Next?

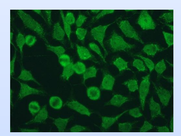

Figure 7: HeLa cells stained with green fluorescent tags

In the upcoming Spring Quarter, we are planning to incorporate real cancer cells into our experiments. We will use HeLa cells from an epithelial cancer line. They are sensitive to epithelial cell adhesion molecules (EpCAM), which are highly prevalent on the surface of epithelial cancer cells. We plan to replace our biotin proteins with HeLa cells and EpCAM proteins, and our Avidin-coated beads with anti-EpCAM coated beads.

References

Figure 1: Tumor Cell http://www.pnas.org/content/110/13/4861/F1.large.jpg
Figure 2: Product Design created in Solidworks 3D CAD Software.
Figure 6: Images taken using an Nikon Eclipse Fluorescent Microscope in the Edwards Lifesciences Center for Advanced Cardiovascular Technology at UCI
Figure 7: Immunofluorescent HeLa Cells
http://www.epitomics.com/images/products/4502-ICC-500_A.jpg

CONTACT US

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