**INTRODUCTION**

Circulating tumor cells (CTCs) are recognized as a powerful indicator for cancer prognosis [1]. Microfiltration is commonly used to isolate and enrich CTCs from blood [2], but the purity of isolated cells is often very low due to filter fouling by leukocytes [3]. We propose a novel microfiltration cartridge (MFC) for live-imaging of filtration and use it to study the purity of isolated cell populations.

**MICROFILTRATION SETUP**

Our custom design for the MFC allows sample to be applied to the bottom of the filter, so that live imaging can occur with an inverted fluorescent microscope (Fig. 2a). The working distances of standard 4x or 10x objectives are compatible with the cartridge. The MFC was laser-cut from acrylic (Aline, Inc.) 1 cm diameter silicon filters (Fig. 2b) containing up to 600,000 uniform pores in 6-20 μm diameter (Fig. 2c) were fabricated by photolithography and deep reactive ion etching. These filters can be sandwiched between the top and bottom layers of the cartridge during assembly (Fig. 2d). O-rings and a silicone gasket are used to prevent leaks. The cartridge is placed on the microscope stage and a syringe pump is used to inject the blood sample (Fig. 2e).

**OPTIMIZING PURITY**

Our experimental setup involves:

1. Collecting mouse blood and staining blood cells red with fluorescent CellTracker™ dye
2. Culturing MDA-MB-231 breast cancer cells and staining green with fluorescent Vybrant™ dye
3. Spiking blood sample with MDA cells
4. Assembling the MFC with selected filter and injecting diluted blood sample with a syringe pump
5. Washing the filter with PBS to reduce contamination by leukocytes

We have identified three parameters which affect the specificity of filtration: the sample dilution, the filter pore size, the flow rate of filtration. Fig. 3 illustrates the extremes of either (a) maximizing efficiency or (b) maximizing purity.

**LIVE IMAGING OF FILTRATION**

With the aid of live imaging, we were able to characterize the effect of the PBS wash on filter specificity. Fig. 4 shows the reduction of background contamination with the increasing volume of PBS wash. We accomplish this without the loss of captured cancer cells.

**ANTI FOULING TECHNIQUES**

Back pulses are typically used in macrofiltration to reduce filter cake formation, however to our knowledge they have not been implemented in microfiltration. Following real-time visualization, we observed that adding two short pulses of back flow during washing can further improve the purity of captured cells (Fig. 5a). Using back pulses, we were able to reduce the contamination by blood cells to below that of both fast flow conditions and previous washing conditions (Fig. 5e).

**CONCLUSION**

We have used a novel MFC for live-imaging the filtration of cancer cells from whole mouse blood in order to optimize filtration and washing steps. Using real-time monitoring with the MFC, we can:

- Increase the purity of trapped cancer cells without compromising recovery
- Explore the use of back pulses to further reduce contamination
- Improve the specificity of simple, rapid filtration

By improving the purity of cells isolated using a label-free method, we can facilitate easier downstream analysis.

**REFERENCES**