Popular Science Summary

What if a doctor could know how a patient with metastatic cancer would respond to the various treatments available? What if there was a way to access the patient's malignant cells without the need for an invasive and painful tissue biopsy? A strategy that would allow access to clinically relevant information but is not causing any increased risk for the patient, and is a feasible, low-cost option available to standard of care. Such measures would act as informative biomarkers, aiding physicians in the patient stratification through the various states of disease progression.

One promising biomarker to address such unmet clinical needs is circulating tumor cells (CTCs). CTCs are malignant cells that have shed from the primary tumor or any metastatic site into the blood stream, where they can be found at an extremely low concentration, approximately 1 to 10 CTCs per milliliter of whole blood. A milliliter of blood contains billions of blood cells and cell fragments of various types (red blood cells (RBCs), different white blood cells (WBCs) and platelets), therefore, it is a tremendous technical challenge to identify and single-out the rare tumor cells. Many isolation methods have been developed over the last decade, all aiming for an efficient assay with high sensitivity and specificity for the target tumor cells. Their approaches vary and are based on both macroscale methods, like cell density-based centrifugation and cell size-based filters, and different types of microscale technologies (*i.e.,* microfluidics) using external forces or passive methods.

In this thesis, a microfluidic technology called acoustophoresis have been explored and optimized for the isolation of CTCs. Acoustophoresis uses ultrasound to manipulate cells and particles in microfluidic channels, *i.e.,* cylindrical, or square channels with micrometer-scaled cross-sections and of centimeter-sized lengths. The applied ultrasound give rise to acoustic forces acting transversely in the channel on suspended cells and particles flowing through. The acoustic force is dependent on the objects size, density, and compressibility, as well as the properties of the suspension fluid. Thereby, the cells and particles can be separated based on their individual acoustic properties.

In paper 1 and 2, live cancer cells from a model system with a prostate cancer cell line added into diluted WBCs were isolated with acoustophoresis. To enhance the separation of cancer cells and blood cells, micrometer-sized elastomeric particles were used to remove the contaminating WBCs. The rubber-like particles behave in the opposite way to cells when impacted by an acoustic wave field. Instead of moving to a pressure-minima, they move to a pressure maximum, here, that is to the sidewalls of the channel. By modifying the particles surface and attaching antibodies that binds specific to WBCs, the particles can act like transporters with the blood cells as cargo. In paper 1, the method was demonstrated in a proof-of-principle study. Paper 2 extended the concept to carry our processing of whole blood. It was done by two-step acoustophoresis, with negative selection acoustophoresis with the modified elastomeric particles as a second step. Future studies contain the processing of patient samples with the objective to isolate and grow patient-derived prostate cancer cells.

Paper 3 studied the cell separation performance of acoustophoresis at increased sample throughputs. The throughput is an important consideration with rare cell separations for clinical use, as a sufficiently large

volume of blood needs to be processed to find the target cells, and also be completed within a reasonable time frame. A phenomenon was discovered at the higher sample throughputs investigated, in which separations failed due to a fluid inertia effect. The gained knowledge can assist in the development of novel acoustofluidic chip designs for high throughput assays.

In paper 4, acoustophoresis was compared to the current state-of-the-art technology for CTC isolation. Two blood samples were drawn from each prostate cancer patient included in the study. The samples were chemically preserved and processed with the two CTC isolation platforms. Image analysis showed that acoustophoresis was able to detect more CTCs with the commonly described CTC characteristics compared to the standard modality. Further, several cells with an alternative CTC feature were also discovered, which would be missed with the current standard due to its isolation approach. Future studies will continue the comparison of CTC-acoustophoresis to the clinical standard technology, including prospective validation of the technology.

The presented work extends the applications of cutting edge acoustophoresis technology in the field of rare cell isolation. The novel approach for CTC isolation may result in a non-invasive methodology to access clinically relevant information to guide management of metastatic cancer patients.