Development of clinical applications based on acoustofluidics.

Purpose & aims

This project aims to develop two new principles for blood component preparation that will fulfil unmet needs in clinical blood diagnostics. The fundamental mode of operation is to A) utilise acoustic standing wave technology in lab-on-a-chip systems to either perform rapid enrichment of bacteria in blood samples by acoustic trapping for immediate PCR analysis, or B) to perform microchip-acoustophoresis-based separation of erythrocytes from leukocytes utilising the intrinsic acoustophysical fingerprint of the cells in optimised buffer systems. The acoustic trapping system **aims at providing diagnostic readout of sepsis samples within one hour** addressing the urgent need for rapid pathogen identification and to immediately initiate antibiotic administration. We will also develop **a rapid point-of-care (POC) method for Chlamydia diagnosis** by rapid acoustic enrichment and purification of the pathogen and PCR readout. The **acoustophoresis-based erythrocyte/leukocyte separation** addresses the need for a fast and simple means to obtain **pure leukocyte populations from blood** samples for subpopulation profiling and genetic analysis. Hitherto unknown physical data on acoustic contrast factor and compressibility linked to density for a range of human cells and cell lines will also be derived and used to calculate separation conditions for a given set of cells. The project will specifically deliver:

- An acoustophoresis-based prototype system for leukocyte purification from blood samples
- Generation of a database of acoustophysical parameters for erythrocytes and leukocyte subpopulations.
- Optimised acoustophysical buffer media properties and a acoustophoresis protocol for leukocyte purification from blood
- A performance study on leukocyte purification performed in a clinical sample set.
- A disposable acoustic trapping based microfluidic system for pathogen extraction from blood and urine samples for rapid microchip PCR diagnostics of sepsis and chlamydia infection
- Integration of a PCR-protocol in an acoustic trap
- A performance study of the pathogen identification system in sepsis and Chlamydia samples

Survey of the field

Sepsis

The incidence of severe **sepsis** in the European Union has been estimated at 90.4 cases per 100 000 population causing **up to 135 000 deaths per year in Europe** (Neilson et al., 2003). The outcome of sepsis depends strongly on early identification and rapid initiation of appropriate antimicrobial treatment against causative organism. Blood culture is currently the golden standard in the microbiological diagnosis of bloodstream infections, but suffers from the long time-to-result, typically 24-48 hours for detection and identification of the pathogen. Newer molecular methods for sepsis diagnostics are based on detection of the bacterial or fungal nucleic acids and the antibiotic-resistance genes. Due to the very low amount of pathogen's genetic material directly available in blood, most of the molecular methods are time consuming and elaborate comprising: 1) enrichment culture of the target organisms from whole blood (or alternatively starting from a large sample volume); 2) concentration and isolation of the genetic material; 3) amplification by broad-range PCR and 4) post-PCR identification of the causative organism and antibiotic-resistance genes by sequencing or microarray techniques (Westh et al., 2009; Wellinghausen et al., 2009). **The most time-consuming step in the present molecular methods is sample preparation**, which at the same time **concentrates the genetic material and eliminates potential inhibitors before the amplification**. This proposal will **address the unmet clinical need to develop a faster sample preparation scheme in enrichment and purification of pathogens in urine and**

blood. The research will capitalise on a microchip-based acoustophoretic platform for bacteria enrichment from whole blood that is under development within the ACUSEP an EU FP-7 work program, which terminates in April 2014.

Chlamydia

Chlamydia *trachomatis* (CT) infection is the most common bacterial sexually transmitted infection (STI) worldwide (Honey et al., 2002. Genital CT infection can cause infertility and ectopic pregnancy (Ahmad-Tajudin et al., 2013). Women infected with chlamydia are up to five times more likely to become infected with HIV, if exposed. C. trachomatis is also an important neonatal pathogen, where it can lead to infections of the eye (trachoma) and pulmonary complications. Effective treatment for CT infection exists, but **lack of simple, fast, reliable, and inexpensive diagnostics complicates the therapy and the prevention of CT transmission**. A high percentage (50-70%) of infected persons remain asymptomatic. Thus, approximately one fourth of patients with positive laboratory test fail to return to clinic for receiving test results and treatment (Westh et al., 2009; Wellinghausen et al., 2009). Automated urine sample preparation methods are available and have decreased assay hands-on time but the overall assay time still remains in hours and are used in combination with PCR only in the specialized central laboratories due to instrumentation complexity. The more rapid point-of-care (POC) tests are based mainly on antibodies. They are rapid, but have low diagnostic sensitivities (50% – 70%) compared to PCR based testing (WHO 2004). **A rapid POC based platform for CT-diagnostics based on pathogen enrichment and purification in disposable acoustic seed-trapping capillaries linked to microchip PCR readout will be developed**.

Leukocyte isolation

Leukocyte/white blood cell (WBC) isolation from blood is one of the most common blood processing procedures in the clinic, where centrifugation still is the ruling method. However, conventional centrifugation only provides a so called buffy coat that holds a significant background of platelets and erythrocytes that interfere with many analytical procedures. In order **to generate pure fractions of leukocytes density gradient centrifugation based on e.g. Ficoll density media addition is required**. **This procedure is costly and typically takes 2 hours of which 35 minutes occupy a skilled lab technician**. Selective lysis of erythrocytes can be employed but the chemical treatment may influence the WBC population and subsequent purification step may be needed. White blood cells are key routine diagnostic targets where analyses of immune function, genetic profile, differential WBC count are some of the most common laboratory activities, performed millions of times every day globally. Based on our understanding of and recent development within microchip-based acoustophoretic cell handling we anticipate the **development of a rapid and easy to use method for isolation of leukocytes from blood to meet the clinical needs and facilitate daily operations in the health care sector.**

Acoustofluidics

Acoustophoresis is a novel separation technology that facilitates handling of cells and particles (larger than 2- 3 micrometers) in microfluidic systems using acoustic forces. The fundamental mode of operation involves the establishment of an acoustic standing wave in the centre of a well-defined microchannel by means of a piezoelectric actuator (Nilsson et al., 2004). Commonly particles/cells that have a density higher than the carrier medium will migrate to the pressure node in the centre of the microchannel under the influence of the acoustic radiation force, F_{Ax}, eq. 1.

$$F_{Ax} = 4\pi \bar{E} R^3 k \sin(2kx)\Phi$$

$$\Phi = \frac{\rho_p + \frac{2}{3}(\rho_p - \rho_0)}{2\rho_p + \rho_0} - \frac{1}{3}\frac{\rho_0 c_0^2}{\rho_p c_p^2} \qquad \text{eq1}$$

ρ_p & ρ_0 are the densities and c_p and c_0 the speed of sound in the particle/cell and the fluid respectively. Φ is the acoustic contrast factor, r - the radius of the cell/particle, $k = 2\pi/\lambda$ and λ the wavelength of sound in the fluid

This way cells can e.g. be concentrated into the centre fraction of a flow channel and thereby be extracted as an enriched population at a multi-port outlet at the end of the channel. Figure 1 shows a schematic cross-section of a microchannel with red blood cells during an acoustic focusing event. The far right shows a confocal scan of the actual distribution of particles focused in the centre of the channel.

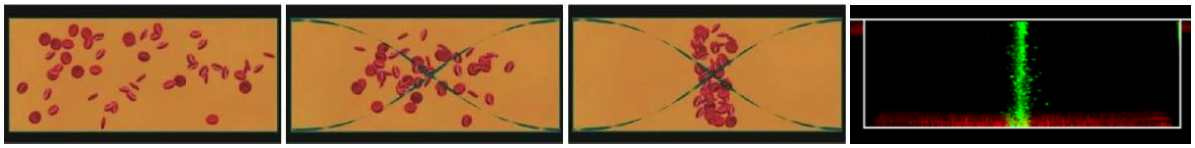

Figure 1 Schematic of an acoustic focusing sequence. Right, a confocal z-scan of focused microparticles

The fact that different particles/cells display individual mobilities in the acoustic force field that is related to their intrinsic physical properties enables the development of microfluidic systems for e.g. cell separation (Petersson et al., 2007). The [redacted] **group is a leading player in the development of chip integrated cell separation systems using acoustic standing wave forces** as the basic means to manipulate cells in microfluidic systems, where concepts for cell washing, concentration and separation have been realised (Persson et al., 2008; Petersson et al., 2005; Nordin and Laurell, 2012) and more lately clinical applications have come into focus where the early efforts of washing recovered blood during major surgery (Jönsson et al., 2004) have been followed by applications in transfusion medicine (Dykes et al., 2011) and cancer diagnostics (Augustsson et al., 2012). It should be noted that the radiation force, F_{ax}, is strongly dependent of the particle size and although cells in general are easily manipulated the radiation force declines rapidly as the size becomes smaller. A general rule of thumb is that particles smaller than 1-2 micrometres are not easily or at all possible to control by the acoustic radiation force. The underlying reason for this is the fact that in an acoustic resonator, operating at its resonance, acoustic streaming will simultaneously be present, which drives rotary flows that counteract the collection of particles in the central pressure node (black vortices, Fig. 1). At the same time as the radiation force drives particles towards the centre of the flow channel the acoustic streaming induced rotational flow (black vortices Fig1.) tends to counter act this and as the particle size gets smaller the streaming force on a particle, Stokes drag, $F_s = 6\rho\rho\eta v$ (ρ–density, η–viscosity, v-velocity, r-radius) becomes significant and typically around 2 µm size (polymer particles) the streaming induced force starts to balance the radiation force and hence acoustic focusing by means of the primary radiation force is hampered (Barkholt-Muller et al., 2012).

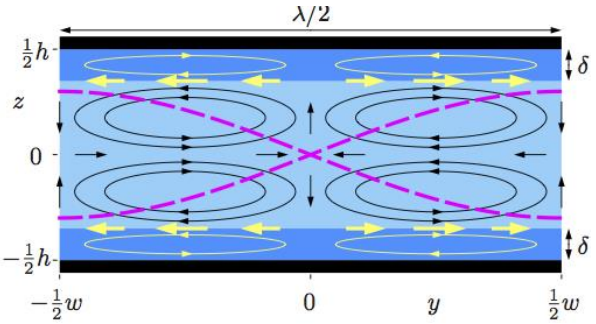

Figure 2. Schematic cross section of a microchannel at λ/2 resonance (purple dashed line) and the obtained acoustic streaming patterns.

An optional strategy to enrich biological material in a streaming media is to employ acoustic trapping in a microfluidic system, which utilises standing wave forces (a combination of the lateral force potential and the radiation force, Fig 3a) in a locally defined acoustic resonator, Figure 3&4. Cells entering the acoustic resonator will be retained in the trapping zone against the flow by the lateral force potential and focused in the centre by the radiation force and hence enrich the species in a non-contact modality which facilitates extraction of the enriched species simply by inactivating the acoustic resonator and collect the enriched sample at the system outlet (Evander et al., 2007). Acoustic trapping can efficiently be employed to particle sizes down to about 1-2 µm, limited by the strong dependence of the primary radiation force that diminishes with reduced particles radius. Hence submicron particles and bacteria cannot be efficiently trapped in a capillary acoustic resonator that is

operated in the standard range of 1-10 MHz without employing the "seed trapping" technology recently discovered by our group (Hammarström et al. 2012), see *Preliminary results*.

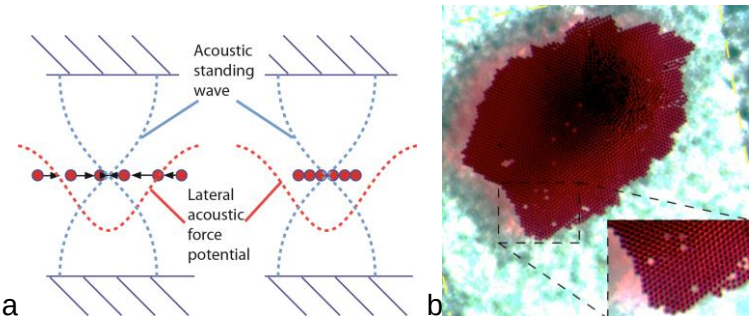

Figure 3a) Schematic of the acoustic trapping principle where the radiation force drives particles into the λ/2 pressure node and the lateral acoustic force potential drives the particles into a dense cluster in the acoustic trapping zone. **b)** A close up of an enriched microbead cluster in an acoustic trap.

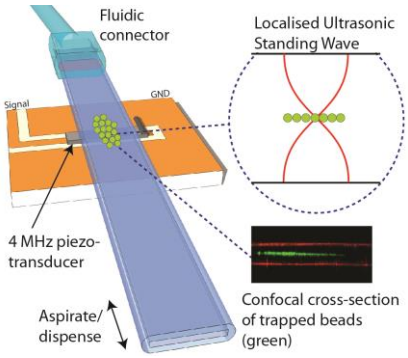

Figure 4. Schematic of capillary based acoustic trap with a micro piezo transducer docked to the capillary and fluorescent (green) beads non-contact trapped in the capillary centre.

Preliminary results.

Sepsis

A key discovery made by the [redacted] group in 2012 (Hammarström et al 2012) was that larger particles can be pre-loaded into the acoustic trap (particle seeding) and retained against flow, and then attract and **trap sub-micrometer particles**. The attraction is due to a secondary acoustic force that originates from sound waves scattered between the particles, large and small. Equation 2 describes the inter particle scattering force, F_{sec}, in the standing wave pressure nodal plane, where: p – pressure amplitude, β_0, β – compressibility of medium and particle respectively, ω – angular frequency. The inter particle force is inversely proportional to the square of the inter particle distance. Based on these findings we have **recently demonstrated efficient acoustic trapping of E-coli with recoveries up to >95% at flow rates of 10µL/min. At higher flow rates, 90-100 µl/min, the recovery dropped to 60%.**

$$F_{Sec} = -\frac{\omega^2 \rho (\beta_0 - \beta)^2}{9d^2} p^2(x)$$

Eq. 2

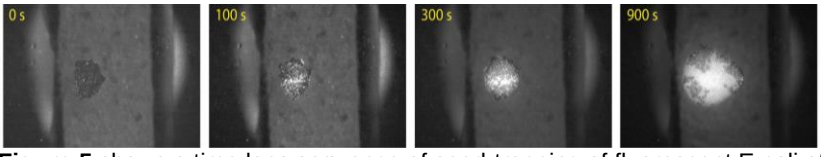

Figure 5 shows a time laps sequence of seed-trapping of fluorescent E-coli at a flow rate of 10 µL/min

Within the EU FP7 funded project ACUSEP (grant agreement no: 259848, finishing in April 2014) we have been developing an acoustophoresis-based system for bacterial enrichment in blood using a pre-separation step of acoustophoretic plasmapheresis (Ahmad-Tajudin et al., 2013) that eliminates the blood cells and provides a cell free plasma that proceeds to the acoustic seed-trapping unit for enrichment of bacteria on a seed-trapping cluster. The trapped bacteria/bead cluster is washed before release it into a PCR microchip, Fig. 6, developed by our collaborators at Turku University, Prof. Tero Suokka. This approach was taken all the way from idea to testing of a prototype with clinical samples within the project. The testing with clinical samples, which ended in March 2014, indicates that the method is indeed capable of detecting bacteria in patient samples, not quite reaching the targeted sensitivity levels of a few bacteria per mL blood. In sepsis, the amount of bacterial load in bloodstream is 1–300 cfu/mL (Chan et al., 2009), which has previously been difficult to detect directly. The system sensitivity for bacteria-spiked samples demonstrates a limit of detection about 10-100 bacteria/mL blood, (Figure 7). The **current project will** now **address the need for a lower limit of detection** based on the experiences made with the ACUSEP-prototype system.

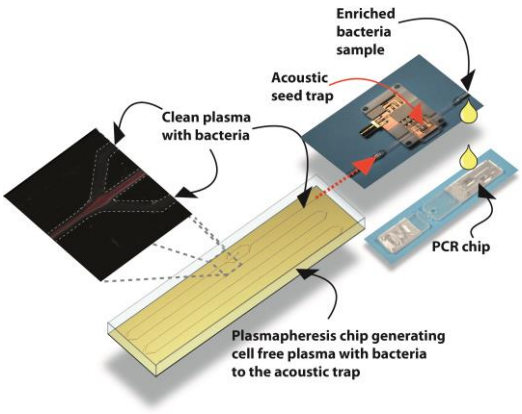

Figure 6. Schematic of the rapid sepsis analysis system developed with the ACUSEP (EU FP7) project. Whole blood undergoes acoustic plasmapheresis (yellow chip) that delivers bacteria in cell free plasma to the acoustic bacteria seed trap that enriches and delivers an aliquot of 50 µL to the PCR chip for bacteria-specific analysis.

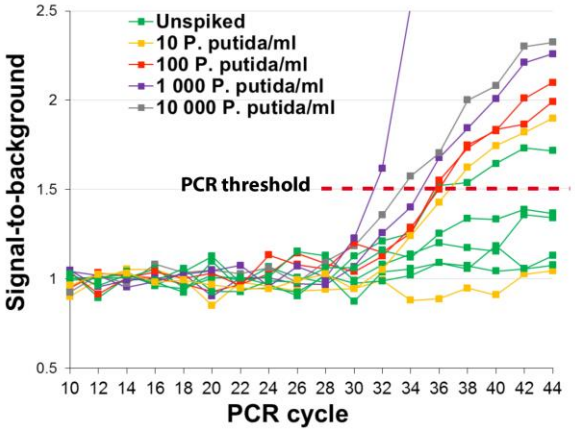

Figure 7 ACUSEP system results from whole blood spiked with 10, 100, 1000 and 10000 bacteria (*Pseudomonas putida*) per mL whole blood. Red dashed line indicates the PCR detection threshold. Levels as low as 10 bacteria/mL have been recorded. One false positive was also recorded in the study.

Leukocyte isolation

Based on our on-going research in acoustophoretic separation of circulating tumour cells, CTC, from leukocytes (WBC) in spiked blood samples we have demonstrated that that cancer cells due to their acoustophysical properties will migrate faster to the centre of the acoustophoresis channel as compared to the leukocytes, Fig. 8. The recent development of an acoustophoresis system that includes a 2-dimensional pre-alignment step of the cells in a uniform velocity vector in the parabolic flow profile to ensure that all cells spends equal time in the acoustophoretic separation zone has vastly improved the system performance (Augustsson et al., 2012). Currently, we deliver high-recovery CTC enrichment with purity against the white blood cell (WBC) background approaching four orders of magnitude (≥92 % tumour cell recovery; <0.03 % WBC contamination, unpublished). This now qualifies the system for evaluation on clinical samples and is work in progress. These findings have triggered the idea of performing high purity WBC/RBC separations in a similar rapid acoustophoresis-based approach. To develop such a system several acoustofluidic aspects need to be considered, see project plan below.

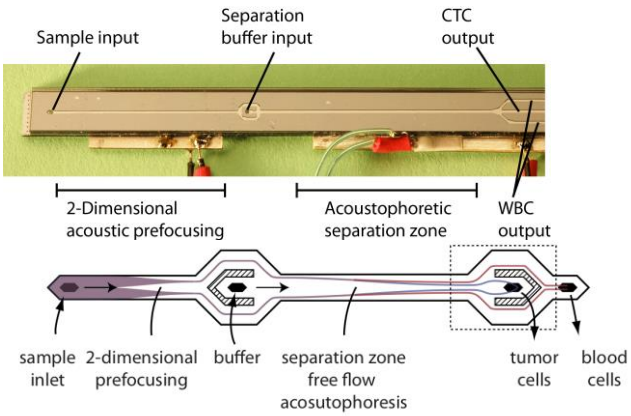

Figure 8. Cell sample enters through the rear inlet (1) and is pre-aligned before reaching the separation channel (3) Clean buffer enters through the 2nd inlet (2). Cancer cells (blue) will be collected at the central outlet (4) whereas blood cells (red) are collected at the rear outlet (5).

Project description

Leukocyte isolation and acoustophysical cell charaterisation

We know that in a standard PBS buffer red blood cells (RBC) will display slightly higher acoustophoretic mobility than WBCs but with overlapping distributions. In order to improve this situation **we hypothesize that the separation conditions can be significantly improved if the proper buffer conditions are employed**, yielding equal data as derived for

CTCs vs. WBCs. Similar to the use of Ficoll in gradient separations where all RBCs migrate to the bottom of the test tube and the WBCs concentrate on top of the Ficoll interface we have preliminary data showing that the mobility of the WBCs can be significantly altered by proper manipulation of the buffer conditions (unpublished). In order to find the optimum buffer conditions for separation we need to know the acoustophysical properties of the cell types to be isolated. This can be performed in a unique measurement system set-up based on particle imaging velocimetry (PIV) recently developed in our lab (Augustsson et al., 2011) that allows measurement of the velocity of particles and cells as they under go acoustophoresis in varying buffer conditions in a microchannel with a calibrated acoustic standing wave pressure field. This yields the acoustic force acting on each cell. Based on this we can subsequently estimate the acoustic contrast factor for each cell type. By **tuning the buffer density and compressibility we anticipate being able to fine tune buffer conditions for WBC/RBC separation that resembles the situation that is accomplished in density gradient separation**. The fundamental principle of the microfluidic system is seen in Fig. 9., where the sample enters the separation along the side inlets and the separation buffer enter the central inlet (2-dimensional prefocusing is not included in the schematic). With a properly tuned buffer only RBCs will be able to pass the boundary between the two fluids under the influence of the acoustophoretic force.

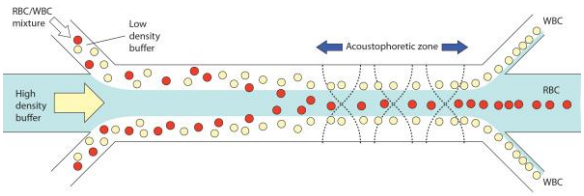

Figure 9 Schematic principle of WBC and RBC separation using an acoustically matched centre buffer that enables only RBCs to pass the interface between the two fluids.

The first step in this process will be to calculate the acoustic contrast factor for RBCs and different types of WBC in under varying buffer conditions based on the PIV data we derive. The contrast factor dependency of each buffer composition for a given cell type can then be used to derive an optimised buffer to be employed in the separation.

By measuring cell size and acoustophoretic velocity in two different buffers with known density and speed of sound, the actual density and compressibility data for each cell type can be derived. This data is currently not available in the literature but yet highly desired as changed mechanical properties of blood cells provide an added diagnostic value. We therefore intend to **derive the first tabulated data on cell compressibility linked to density data as well as the acoustic contrast factor** for each cell type.

Medically approved buffers for media manipulation that includes both density and compressibility variations will be used to enable a clinical acceptance for the method to be developed. A starting point for this work will include combinations of Percoll, Ficoll and standard buffers. This part of the project will as its endpoint aim at finding the best buffer conditions for performing high purity WBC/RBC separations and to generate the first tabulated data on blood cell compressibility, density and the corresponding acoustic contrast factor.

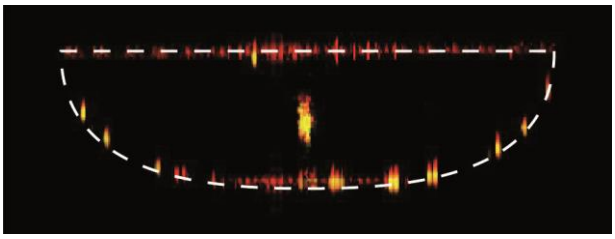

Figure 10. Confokal z-scan of the particle distribution in a 2-dimensionally actuated glass microchannel, demonstrating 2D-focusing.

In order to make the RBC/WBC separation technology industrially attractive we also foresee the **translation of the current silicon chip design into an all glass microfabricated device**. We have already in other studies experienced that the most critical part, i.e. the 2-dimensional pre-focusing, can indeed be realised in an isotropically etched low cost glass device, Figure 10. This part of the project will end with the delivery of a glass microchip RBC/WBC separation prototype system.

Activities

Year 1.

- Developing the PIV measurement method and Mathlab scripts to enable efficient data extraction of acoustic contrast factor, compressibility and cell density directly based on image data
- Validation of the data extraction method on calibration microparticles using the standard silicon microchip based system available

Year 2

- Acoustic contrast factor measurement data on lymphocytes, monocytes, granulocytes and erythrocytes in standard buffers such as PBS.
- Acoustic contrast factor including variability established for WBCs and RBC from healthy donors, measured in a range of buffers with different density and compressibility
- Acoustic contrast factor established for lymphocytes, monocytes, granulocytes and erythrocytes from unhealthy donors

Year 3

- Based on acoustophysical cell data a buffer composition that enables separation of RBC and WBC will de calculated and experimentally investigated.
- Tabulated data on compressibility, density and acoustic contrast factor for blood cell as well as a range of standard cell lines.
- Design and manufacturing of a glass chip based RBC/WBC separation unit with 2-dimensional prefocusing

Year 4

- Evaluation of optimised RBC/WBC separation protocol in a clinical sample cohort. using the silicon microchip platform
- Evaluation of the 2-dimensional prefocusing glass chip cell separator on mixture of calibration particles

Year 5

- Evaluation of the optimised RBC/WBC separation protocol on clinical samples using the glass chip separator.
- Validation that the WBC sub-populations are not biased by the acoustophoretic separation method.

Rapid bacteria analysis - Sepsis

The current status of the ACUSEP project is a hybrid system where the acoustic plasmapheresis chip is connected through a microtube to the acoustic trapping chip (red dashed arrow Fig. 6). The starting material is 1 mL whole blood diluted to 70% in the plasmapheresis process. 180 µL of cell free plasma is generated for the bacteria trapping sequence, which corresponds to 120 µL undiluted blood plasma. In Figure 7 we show that we can detect *P. Putida* at 100 bacteria /mL and in one of two cases we also see 10 readout at 10 bacteria/mL. Given that we only analyse ≈ 1/10 of a mL the chance of finding a bacteria at levels down to tens of bacteria per mL or below becomes a statistical issue. The reason for not running a larger plasma sample volume through the bacteria trapping unit is that the platelet depletion in the plasmapheresis unit is not perfect and most importantly it varies significantly with the patient. Figure 11 shows two cases after performing the bacteria trapping sequence, where a) had $6x10^9$ platelets/mL and b) had $208x10^9$ platelets/mL. In Fig 11b the seed-trapping cluster also displays a massive aggregate of platelets (white) to the left. In case b) we were not able to release and analyse the trapped cluster due to the overload of platelets in the bead cluster, whereas case a) worked fine.

Figure 11 a) Seed trapping completed with a sample having $6x10^9$ platelets/mL

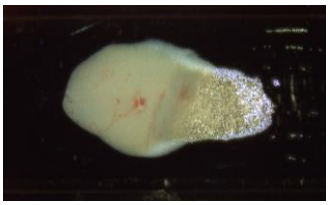

Figure 11 b) Seed trapping completed with a sample having $208x10^9$ platelets/mL

We also experienced that some patient samples displayed platelets that were highly activated and sticky which also compromised the trapping procedure. It stands clear that the seed trapping technology in principle can enrich bacteria directly from samples as dilute as in sepsis cases but **the interference from platelets are currently a major hurdle** to advance the technology into a full clinical study. This part of the project will thus seek ways to **eliminate the background interference from the platelets**. We have two main routes that will be investigated. The first step is to investigate several different lysis protocols both commercial lysis kits as well as standard laboratory lysis protocols. We know from preliminary experiments in other cell-based studies using FACS analysis that some of the more standard lab protocols for blood sample lysis leave significant amount of cellular debris (membrane fragments – ghost cells, intra cellular fragments etc.) that may interfere with the trapping module. We will **investigate different whole blood lysis protocols using FACS analysis and screen the best of these against patient samples** of varying disease background (i.e. varying levels of platelet activation). After the FACS screening we will perform **bacteria seed-trapping on lysed whole blood samples**, using a selected set of lysis protocols, followed by the microchip PCR readout. Initially the studies will be performed on healthy donor blood spiked with known levels of bacteria. This will be followed by studies on clinical samples and benchmarked versus the clinical routine. As an alternative we will also perform the **corresponding study using cell free whole blood plasma after pelleting** the cell components **in an initial centrifugation step**. The benefit of the lysis route is anticipated to be an efficient removal of cellular background interference but on account of an added process cost due to the lysis chemicals. The centrifugation step offers a simple and straightforward route to minimise the platelet interference but loss of bacteria in the centrifugation step is at risk both as bacteria in solution and as cell membrane bound entities. If necessary the investigation will include the plasmapheresis step for a further platelet reduction prior to the bacteria trapping step.

Chlamydia diagnostics

The outlined bacteria **seed-trapping platform will also be investigated as a Point-Of-Care (POC) proof of concept for *Chlamydia* diagnostics**. We will initially make tests by running urine samples directly into the seed-particle trapping unit as the cell background and complexity of urine is rather low. Since our initial testing of acoustic trapping without seed particles gave weak positive signals in about 50% of the positive urine samples we are confident that the seed trapping technology will dramatically improve this situation. The acoustofluidic POC-system will be evaluated on urine samples tested for *Chlamydia* infections. Samples will be obtained through the centralised laboratory at UMAS, Region Skåne. We have several hundred *Chlamydia*-specific PCR microchips available in our lab through our collaboration with Prof. [redacted] Univ. Finland. The testing will be performed at the Dept. of Laboratory Medicine, Clinical Microbiology, Lund University Hospital, in collaboration with Dr. [redacted] who will be in charge of the sample collection logistics and reference readouts. Benchmarking is made versus the clinical routine and an initial evaluation will be performed on 100 de-identified samples: 50 positive and 50 negative controls.

Activities

Year 1

- Pilot study on the *Chlamydia* POC – system
- Study on different lysis protocols based on FACS assaying for acoustic trapping based sepsis analysis

Year 2

- Study of acoustic seed trapping using a selected set of lysis protocols on healthy donor blood samples spiked with know bacteria levels
- Study of the Chlamydia POC system in a clinical cohort of 100 samples

Year 3
- Study of the acoustic seed trapping bacteria enrichment on a small clinical sample set, using the selected lysis protocol

Year 4
- Centrifugation protocols for cell free plasma and initial acoustic seed trapping of bacteria in spiked healthy donor samples

Year 5
- Comparative study of acoustic seed trapping for sepsis samples either using centrifugation or lysis for platelet removal

Significance
The value of a fast, low cost and easy to use method for unbiased white blood cell isolation cannot be over emphasized. Sub-population differentiation and genetic analysis of WBCs are major routine diagnostics in the daily health care flow. Rapid isolation of WBCs also finds a great need in preclinical and bioanalytical research where access to an operator independent method for WBC purification would be highly valued. The establishment of a data based comprising acousto mechanical and physical data that have not been available before will open up for new data correlations in disease investigations and to study the physiological conditions of cells in different biological states. Acoustofluidic enrichment of microparticles/cells and submicroparticles offer an advantage over conventional techniques where either extensive centrifugation and or filtering processes are employed to enrich the target species. These are time-consuming techniques that involve extensive manual labour and in case of mechanical filters the risk of clogging is significant. The proposed **acoustic seed-trapping enrichment strategy offers a filter-free mode of operation** and can be implemented in a continuous flow-based system, hence enabling on-line integration with existing analytical instrumentation/techniques and thus automated operation. In the case of bacterial analysis there is a need for a rapid mode of enrichment and pre-conditioning for PCR analysis. One of the major bottlenecks in current protocols for rapid bacteria identification by means of PCR is washing of the sample by multiple centrifugation steps or bacteria culturing over days. The described **acoustic seed-trapping system will intrinsically offer both rapid enrichment and sample washing in one unit** without operator intervention prior to direct PCR analysis. Our preliminary data and the current research proposal describe **a generic solution to the enrichment and pre-processing of urine, blood samples and other biofluids in medical diagnostics**.

Equipment
The Laurell laboratories have in-house microfabrication facilities for the development of the outlined microfluidics chips at LTH. The laboratory is well equipped with fluorescence and confocal microscope resources as well as an acousto-microfluidics laboratory with a unique set-up for particle imaging velocimetry and measurement of the acoustic fields in acoustic resonators. The Laurell group also have laboratory facilities at the Biomedical Centre in Lund where flow cytometers and Coulter counters are in place for the characterisation of acoustofluidic separations as well as cell culture laboratories and indoor access to the clinic. The collaboration with the Dept. of Laboratory medicine, Clinical Microbiology, is only a sky bridge away.

International and national collaboration
EU FP7 ITN PEPMIP (' [redacted] Tech. Univ. Dortmund, Germany); **SSF-Japan** Multidisciplinary BIO ([redacted], Tokyo Univ., Japan); **EU FP7** ACUSEP ([redacted] Finland); **FORMAS** [redacted] **VINNOVA:** Innovation for Future Health [redacted]-

[redacted]); **Knut och Alice Wallenbergs Stiftelse** – [redacted], **Prof.** [redacted] Dept. of Microbioanalytics, Warsaw Univ. Tech., Warsaw, Poland, [redacted] Research Environment [redacted]; STINT [redacted] Dongguk Univ., Seoul, Korea - [redacted] KTH - [redacted] - automated quality control of [redacted] Medical Microbiology, University Hospital Lund, clinical studies of bacterial enrichment in microfluidic systems.

Ethical considerations

We will handle biological samples (blood, urine) from patients and healthy volunteers. The samples are anonymised when we get them. Current ethical permits for routine investigations will be utilised, in collaboration with the participating clinics. We use only a small part of samples that are taken routinely in these clinics.

References

Augustsson P., et al. **Anal Chem,** 2012, 84, 7954-7962 dx.doi.org/10.1021/ac301723s
Augustsson P. et al. **Lab Chip,** 2011, 11 (24), 4152 - 4164
Ahmad Tajudin, A. et al. **Lab Chip**, 2013, 13 (9), 1790 - 1796
Barkholt Muller P., et al. **Lab Chip**, 2012, 12, 4617–4627
Chan, K. Y. Y. et al. Crit Care Med 2009 (37):2441-2447.
Dykes, J. et al. **PLOS One**, 2011, 6, 8, e23074
Evander, M. et al. **Anal Chem**, 2007, 79, 2984-2991
Hammarström B, et al. **Lab Chip**, 2010, 10, 17, 2251-2257
Hammarström B., et al. **Lab Chip**, 2012, 12, 4296-4304
Honey, E., et al. **Sex Transm Infect**. 78, 406-412, 2002.
Jönsson H., et al. **Annals of Thoracic Surgery**, 2004, 78, 5, 1572-1578
Neilson, A. R. et al. J Crit Care 2003 (18):217-227.
Nilsson A., et al.**Lab Chip**, 2004, 4, 131 – 135
Nordin M., and Laurell T., **Lab Chip**, 2012,12 (22), 4610 - 4616
Petersson F. T., et al. **Anal Chem**, Accelerated article, 2007, 79, 14, 5117-5123.
Persson J., et al. **FEBS J**, 2008, 275, 5657-5666, Journal cover illustration
Petersson F., et al. **Anal. Chem**, 2005, 77, 5, 1216-1221, Accelerated Article
Wellinghausen, N. et al. J Clin. Microbiol 2009 (47):2759-2765.
Westh, H. et al. Clin Microbiol Infect 2009 (15):544-551.
"WHO Disease and injury country estimates". World Health Organization. 2004. http://www.who.int/healthinfo/global_burden_disease/estimates_country/en/index.ht ml. Retrieved Nov. 11, 2009.