LUND UNIVERSITY

On acoustic particle and cell manipulation in microfluidic systems

Petersson, Filip

2007

Citation for published version (APA):
Petersson, F. (2007). *On acoustic particle and cell manipulation in microfluidic systems*. Department of Electrical Measurements, Lund University.

Total number of authors:
1

On acoustic particle
and cell manipulation
in microfluidic systems

Filip Petersson

Department of Electrical Measurements
Lund Institute of Technology
Lund University

On acoustic particle and cell manipulation in microfluidic systems

Filip Petersson

LUND UNIVERSITY

Organization: Lund Institute of Technology, Lund University Department of Electrical Measurements P.O. Box 118, S-221 00 Lund, Sweden	Document name: Doctoral Dissertation
	Date of issue: May 11, 2007
	Sponsoring organization: The Swedish Research Council (Vetenskapsrådet)
Author: Filip Petersson	

Title: On acoustic particle and cell manipulation in microfluidic systems

Abstract:

The combination of laminar flows in microfabricated channels and acoustic forces induced in ultrasonic standing wave fields offers new possibilities for advanced particle and cell manipulation in lab-on-a-chip applications as well as in relatively high throughput applications. Acoustic particle manipulation systems can be used e.g. to separate, wash, sort, trap or distribute particles in microfluidic networks. This doctoral thesis starts by reviewing the fundamental fields of study needed to realize such systems and to understand their potential impact. Next, the design, fabrication, operation and performance of a number of systems, based on system design principle termed "the Lund method", are described. The developed methods comprise a toolbox of generic particle handling methods that can be combined or used separately to handle biological and non-biological particles in liquid suspension. The toolbox is applied to solve various blood component handling tasks, e.g. separation of lipid particles and other contaminating substances from red blood cells and preparation of blood components. The results imply that it is possible to save thousands of people from brain damage caused by lipid particles each year, to reduce the strain on the blood banks significantly and to offer new methods for routine blood component handling. Several other important areas of application, where micrometer sized particles are routinely handled, can also be identified. Microscale acoustic particle manipulation technology is still in its infancy but, based on the findings presented in this thesis and by other researchers, it can be anticipated that new laboratory and industrial standards may very well emerge from the current research.

Keywords:

microtechnology, microfluidics, microfabrication, ultrasound, lab-on-a-chip, μ-TAS, separation, cells, microparticles, acoustic particle manipulation, standing wave, acoustic radiation force, laminar flow

Classification system and/or index terms: UMI Subject Category 0605

Supplementary bibliographical information: ISRN LUTEDX/TEEM - - - 1085 - - - SE, Report 1/07	Language: English
ISSN and key title: 0346-6221	**ISBN:** 978-91-628-7144-4

Recipient´s notes:	**Number of pages:** 130	**Price:**
	Security classification:	

To my grandmother

Cover illustration
Illustration showing the separation of two dissimilar particle types by means of the axial primary radiation force in a standing wave field.

Public defence
May 11, 2007, 10.15 in E:1406, E-huset, Lund Institute of Technology

Advisors
Professor Thomas Laurell and Dr Monica Almqvist
Department of Electrical Measurements, Lund Institute of Technology, Lund University, Sweden

Faculty Opponent
Dr Martyn Hill
Department of Mechanical Engineering, School of Engineering Sciences, University of Southampton, United Kingdom

Board of Examination
Professor Hans Hertz
Department of Biomedical and X-Ray Physics, Royal Institute of Technology, Sweden
Dr Anders Christensson
Department of Nephrology and Transplantation, Malmö University Hospital, Malmö University, Sweden
Professor Anders Axelsson
Department of Chemical Engineering, Lund Institute of Technology, Lund University, Sweden
Deputy member: Dr Jonas Tegenfeldt
Department of Solid State Physics, Lund Institute of Technology, Lund University, Sweden

List of included publications

I   **Acoustic control of suspended particles in microfluidic chips**
Andreas Nilsson, Filip Petersson, Henrik Jönsson and Thomas Laurell
Lab on a chip, 2004, 4, 131-135

II   **Separation of lipids from blood utilizing ultrasonic standing waves in microfluidic channels**
Filip Petersson, Andreas Nilsson, Cecilia Holm, Henrik Jönsson and Thomas Laurell
Analyst, 2004, 129, 938-943

III   **Particle separation using ultrasound can radically reduce embolic load to brain after cardiac surgery**
Henrik Jönsson, Cecilia Holm, Andreas Nilsson, Filip Petersson, Per Johnsson and Thomas Laurell
Annals of Thoracic Surgery, 2004, 78, 1572-1578

IV   **Carrier medium exchange through ultrasonic particle switching in microfluidic channels**
Filip Petersson, Andreas Nilsson, Henrik Jönsson and Thomas Laurell
Analytical Chemistry, 2005, 77, 1216-1221

V   **Free Flow Acoustophoresis (FFA) – a new microfluidic based mode of particle and cell separation**
Filip Petersson, Lena Åberg, Ann-Margret Swärd-Nilsson and Thomas Laurell
Submitted, 2007

List of related publications

A   **Continuous separation of lipid particles from erythrocytes by means of laminar flow and acoustic standing wave forces**
Filip Petersson, Andreas Nilsson, Cecilia Holm, Henrik Jönsson and Thomas Laurell
Lab-on-a-chip, 2005, 5, 20-22

B   **Particle separation using ultrasound can be used with human shed mediastinal blood**
Henrik Jönsson, Andreas Nilsson, Filip Petersson, Mats Allers and Thomas Laurell
Perfusion, 2005, 20, 39-43

C   **Chip integrated strategies for acoustic separation and manipulation of cells and particles**
Thomas Laurell, Filip Petersson and Andreas Nilsson
Chemical Society Reviews, 2007, 36, 492-506

Table of contents

List of abbreviations

CFD   Computational Fluid Dynamics
CVD   Chemical Vapour Deposition
DC   Direct Current
DRIE   Deep Reactive Ion Etching
DSMC   Direct Simulation Monte Carlo
EDP   Ethylenediaminepyrocatechol
ESZ   Electrical Sensing Zone
FACS   Fluorescence Activated Cell Sorting
FFA   Free Flow Acoustophoresis
FFF   Field Flow Fractionation
IC   Integrated Circuit
KOH   Potassium hydroxide
LDV   Laser Doppler Velocimetry
MD   Molecular Dynamics
MEMS   Micro Electro Mechanical Systems
MST   Micro System Technology
MTV   Molecular Tagging Velocimetry
PIV   Particle Image Velocimetry
PRF   Primary Radiation Force
ODT   Optical Doppler Tomography
RIE   Reactive Ion Etching
SIV   Scalar Image Velocimetry
SPLITT   Split Flow Thin Fractionation
SRF   Secondary Radiation Force
TMAH   Tetramethylammonium hydroxide
UV   Ultraviolet
μ-TAS   Micro Total Analysis System

1. Introduction

1.1 General background

Everything started with mixing that went wrong and became sorting instead. Some years ago a couple of engineering students got the task to use ultrasound to mix particles and a liquid within a small channel. To everyone's surprise they found that, instead of being mixed, the particles were aligned in well defined rows along the length of the channel. Since this result was the opposite of what they were trying to achieve the whole thing was regarded as a failure at the time. However, experimental results, good or bad, should always be remembered and one day the failed mixing experiments became actualized again. When my supervisor, Thomas Laurell, heard that fat particles in blood are believed to cause brain damage during cardiac surgery he suggested that the particle alignment phenomenon might be used to separate the fat particles from the blood. Two master theses later it had been proven possible. Since then, a company called ErySave AB has been formed to develop a blood recycling device based on the findings whilst the continued scientific work has resulted in several additional master theses and, with this work, a doctoral thesis.

The discovery that particles can be affected by acoustic forces induced in standing waves is certainly not new. The phenomenon has been known at least since the end of the 19th century [1]. Generations of scientists have made efforts to understand the nature of these forces and use them in various applications. Nevertheless, the research field has never been particularly large and very few practically useful results have come out of it. Fortunately, an inspiring increase in activity has been seen during the latest decades and at the present time there are several active research groups in the world. Though, many of the presented papers in the field are quite similar in their approach and some fundamental problems associated with the use of acoustic forces in particle handling remain to be solved. In order to understand the phenomena better and to eventually make acoustic particle handling methods widely available, new points of view are essential. Since the work presented in this thesis more or less started from scratch with no prior bias on how acoustic particle manipulation should be carried out, with new fabrication methods at hand and with new applications in mind it can hopefully offer just that.

One might find it strange that a department named "Electrical Measurements" houses a group of scientists that use ultrasound to handle particles in microscale devices with the intention to use the results mainly in medical and biological applications. Though, with the historical background of the department in mind it is not that strange. The tradition in ultrasonics and medicine started in the 1950s when Edler and Hertz discovered that ultrasound can be used for medical diagnostic purposes [2, 3]. As the years went by their findings developed into a standard method that is used at almost every major hospital in the world today. Furthermore, pioneering works in ink jet printing technology started a tradition of fine mechanics and fluidics that also has lived on. More recent additions to the activities at the department include the development of devices for applications in proteomics and neurology.

1.2 A paradigm shift

The term microtechnology refers to technology with features between one millimeter, i.e. one thousandth of a meter, and one micrometer, i.e. one millionth of a meter. The possibilities offered by this size regime were anticipated by Richard Feynman in a talk given in 1959 [4]. Though, it was in the 1960s, when scientists wanted to fabricate chip based microscopic electronic components, that the methods that constitute the foundation of this leap in technology were developed. Later, it was realized that the same methods were suitable for fabrication of mechanical and fluidic devices, which is why microdevices often are categorized as microelectronic (e.g. transistors, resistors and diodes), micromechanic (e.g. gears, motors and bearings) or microfluidic (e.g. valves, pumps and channels). Systems that combine micromechanics with microelectronics are often referred to as Micro Electro Mechanical Systems (MEMS) [5]. Micromechanics, micromechatronics and micromachines are similar terms used in the literature. A more general and modern term that includes all types of microsystems is Micro System Technology (MST) or simply microsystems. The most famous microtechnological success so far is the integrated circuit (IC), i.e. miniaturized electrical circuits on semiconductor chips that triggered the information technology revolution. Nowadays, the turnover of the IC industry is in the order of hundreds of billions of euros annually. The microsystem industry, excluding the IC industry, has an annual turnover in the order of tens of billions of euros and is growing very fast. Typical products are dispensers for ink jet printers, hard disk drive heads, accelerometers for airbag deployment, digital micromirror devices for video projectors, microphones and switches for optical communication. The three pioneers Zhores Alferov, Herbert Kroemer and Jack Kilby were awarded the 2004 Nobel Prize in physics for their contributions in the development of microelectronics.

Microfluidics emerged as a new research field in the 1970s when microsystems for gas and liquid flows were developed. Pioneering works included capillary columns for chromatography and ink jet nozzles [6-9]. However, the real breakthrough came in the early 1990s with the development of chip integrated capillary electrophoresis and since then the research field has grown and branched dramatically [10]. Recently, fluidic systems with dimensions smaller than 1 micrometer have been presented, thereby opening the route to nanofluidics [11]. Well developed microfluidics is a prerequisite for the development of micro total analysis systems (μ-TAS), also known as lab-on-a-chip systems. The basic concept was presented in the beginning of the 1990s as an idea to integrate a typical room sized laboratory on a microchip [12]. In a μ-TAS, analytical processes, e.g. sampling, sample transport, sample preparation, sample injection, sample manipulation, separation, chemical reactions and detection, take place in a sequential manner. The advantages offered by these systems are many, e.g. low reagent and power consumption, short reaction times, potential in situ use, low cost, versatility in design, increased throughput through parallelization and the possibility of integration with other microdevices.

Suspended particles, e.g. cells and different kinds of beads, with or without active surfaces, are common in many research fields. In microfluidic systems, e.g. magnetic, mechanical and electrical methods are generally used to handle these particles [13-15].

Though, handling of suspended particles by means of acoustic forces is an area that is gaining increased attention, both in macroscale and recently also in microscale [16, 17]. A driving factor in the latter case is the simultaneous progress in microfabrication and microfluidics, which now offers precision engineering of acoustic resonators as an integrated part of microfluidic networks. Acoustic methods offer a non-contact mode of particle handling, making it an attractive tool in cell handling microsystems as a minimum of mechanical stress is induced. Since the blood recycling idea was born it has become evident that the potential of acoustic particle handling reaches far beyond removing fat particles from blood. To be more precise, it can be regarded as a generic method for handling of microparticles in liquids. Since this is done on a regular basis in e.g. the biological and medical sciences the potential areas of application are many and the demand for new methods is extensive. The microscale format also offers the exciting possibility to integrate the devices with other microdevices to form complete lab-on-a-chip systems.

1.3 Aims

The primary aim of this thesis work was to combine microfabrication, microfluidics and acoustic forces in order to separate microparticles in liquid suspension from each other and/or from their suspending medium in a format that can be used both as an integrated part of a lab-on-a-chip system and in higher throughput applications. This was to be done through the development and experimental exploration of the acoustic particle manipulation device design principle termed the "Lund method" (described in chapters 6 and 7). The secondary aim was to investigate the applicability of the findings in blood cell handling applications.

The first part of this thesis (chapters 1-9) aims to give a reader with basic knowledge in the engineering sciences an introduction to the relevant fields of study needed in order to understand the included papers and their implications. However, it is my belief that any reader that finds the topics of this thesis interesting will take some pleasure in reading it.

2. Microfabrication

In order to realize microsystems, high precision fabrication methods are needed. These must be capable of generating structures that range in size from micrometers to millimeters, i.e. three orders of magnitude. Such methods were initially developed in the semiconductor industry for microelectronics but are now being used to fabricate a wide variety of microsystems. Old methods have been further developed and new methods, e.g. soft lithography and deep reactive ion etching, have been added to the list.

Logically, microfabrication should only be used when it improves an existing device or allows for new devices to be realized [18, 19]. There are, however, a number of situations when this is the case. First of all, small volume and low weight is often in itself an advantage when a device is to be placed in a confined space or transport costs are high, e.g. in the space industry. Secondly, microfabricated structures offer a high surface area to volume ratio. Thus, surface effects dominate over volume effects, which is beneficial when e.g. surfaces work as catalysts. Thirdly, since basically the same fabrication methods can be used for electronic, mechanical and fluidic microsystems, integration of the three categories of devices is possible. Fourthly, the throughput can sometimes be increased compared to macrosystems due to e.g. shorter analysis times as a result of short diffusion distances and the possibility of massive parallelization. For example, an array chip for analysis of biological samples can hold tens of thousands of active sites, making the analysis extremely efficient. Fifthly, the small sample volumes needed in a microsystem offer a number of advantages. Costs are kept down, the use of individual samples is extended and it is possible to analyse samples that are too limited in volume to be analysed using macrosystems. Sixthly, microfabrication offers extremely good precision in the geometry and placement of microstructures. Today, the resolution has been pushed down to the nanometer level, one billionth of a meter, opening up a new research field referred to as nanotechnology [20-23]. Lastly, the benefits of batch fabrication are extensive since large numbers of identical devices can be fabricated quickly and cost efficiently.

There is a wide variety of microfabrication methods available when a microsystem is to be realized. These are categorized as either subtractive or additive [24]. A subtractive method removes material e.g. through wet or dry etching. Additive methods, on the other hand, add material to a structure through deposition, either as thin films on top of the substrate or as modifications of the surface layer of the substrate. Microfabrication methods can also be categorized as surface or bulk micromachining [5]. Bulk micromachining is the selective removal of parts of the substrate while surface micromachining is the deposition or removal of layers on top of the substrate.

The fabrication of a microsystem involves a sequence of process steps. Some frequently used methods, generally included at least once in these sequences, are discussed in the subsections of this chapter. More details concerning these methods and others can be found in the literature [25-32].

2.1 Choice of substrate

A very common substrate material in microfabrication is monocrystalline silicon, a heritage from microelectronics for which most microfabrication processes initially were developed. Monocrystalline silicon can be produced using the Czochralski technique where a monocrystalline seed crystal is immersed in a melt of polycrystalline silicon after which it is pulled out slowly to create a rod of monocrystalline silicon [26]. An alternative production method is the float zone technique where a rod of polycrystalline silicon with a seed crystal in one end is passed through a ring shaped heater to create a travelling molten zone. When the heater has passed and the melt is cooled down a monocrystalline rod, initiated by the seed crystal, is formed [26]. The monocrystalline rods are then cut into round wafers measuring from tens of millimeters up to hundreds of millimeters in diameter and from a few hundreds of micrometer to about one millimeter in thickness. Monocrystalline silicon has suitable electrical and mechanical properties for microfabrication, i.e. its semiconductor properties and crystal lattice structure offer a wide variety of possibilities. The main disadvantages associated with silicon are that it is rather expensive, it is not optically transparent and it has a low electrical breakdown voltage. Crystalline silicon wafers are available in cuts of various orientations defined by the Miller indices (figure 2.1), e.g. {100}, {110} and {111} [27]. The orientation is of major importance when anisotropic wet etching is utilized but less important when isotropic wet etching or dry etching is employed.
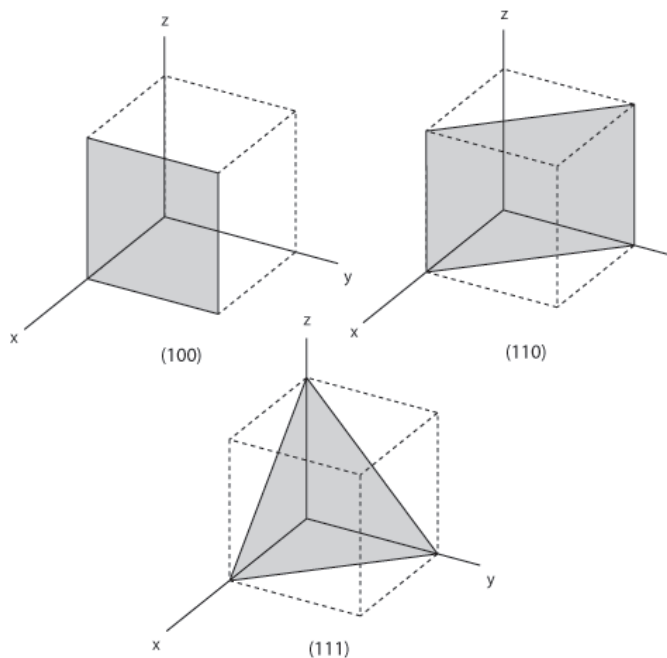
Figure 2.1: *The fundamental crystal planes of monocrystalline silicon and their respective Miller indices. The indices of a plane is defined as the reciprocal of its intercepts with the three Cartesian coordinate axes, in terms of the lattice constant, reduced to the smallest three integers having the same ratio.*

Glass is also a commonly used substrate material. There are several types of glass wafers available, e.g. fused silica (pure amorphous silicon dioxide) and borosilicate (Pyrex) wafers [28]. The advantages associated with glass wafers are e.g. that they are cheaper than silicon and optically transparent. However, because of the amorphous nature of glass some process methods cannot be utilized.

In applications where low cost is of major importance, e.g. in disposable devices, polymer wafers can be used [18]. Microfabrication based on plastic wafers generally produces inferior results compared to silicon or glass since plastics are soft and therefore offer poorer dimensional tolerance and stability.

In addition to the above mentioned substrate materials, more uncommon substrate materials are sometimes used, e.g. ceramics and metals [33-35].

Silicon wafers of orientation {100} and {110} with a diameter of three inches and a thickness of ~270 μm or ~360 μm were used in the work presented in this thesis. Silicon was chosen since its crystal lattice structure offers the possibility to fabricate flow channels with very smooth and parallel side walls through anisotropic wet etching (figure 2.2).
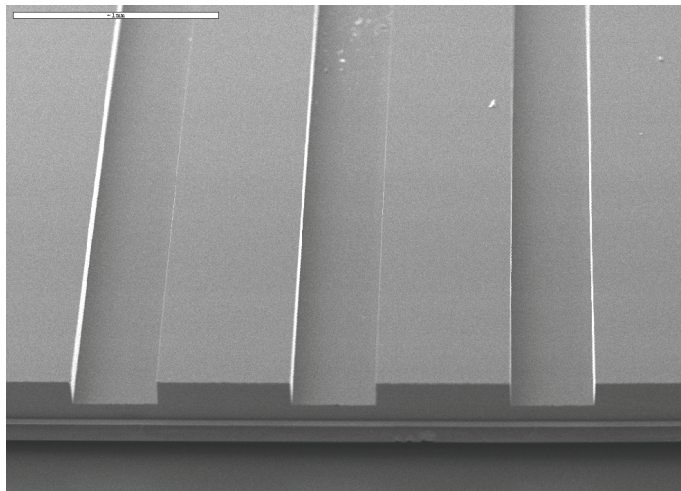
Figure 2.2: *A scanning electron micrograph showing the perfectly vertical and parallel opposing side walls of three anisotropically wet etched flow channels in monocrystalline silicon.*

2.2 Deposition of thin films

When it is desired to cover the surface of a substrate with a layer of a different material, deposition processes are utilized. There are two broad categories of these processes, physical (e.g. evaporation and sputtering) and chemical (e.g. plating and chemical vapour deposition [CVD]). In the latter category, a chemical reaction takes place when the layer is formed. Physical deposition processes, on the other hand, rely on mechanical and thermodynamic effects. Deposited thin films of several types of materials are used in numerous applications in microfabrication, e.g. as masking layers,

structural material, sacrificial material and parts of electrical components [18]. Dielectrics, i.e. electrical insulators, are commonly used thin films [26]. Silicon dioxide, for example, can act as an electrical insulator in electrical circuits or as an etch mask. Silicon dioxide is generally thermally grown through oxidation of the surface of a wafer at temperatures between 900°C and 1200°C under a continuous flow of oxygen or water vapour. Since the chemical reaction slows down as the oxide layer grows, layers thicker than ~2 µm are normally not grown [27]. Deposition of other dielectrics, e.g. silicon nitride, can be done through CVD. However, dielectric films deposited in this way tend to be less robust than thermally grown. In addition to the frequently used dielectrics, several other thin film materials are used. Polycrystalline and amorphous silicon, often used as structural material, can be deposited using e.g. CVD [25, 36]. Metals, e.g. good conductors like gold and platinum, acting as parts of electrical components or as electrodes, are either physically deposited or electroplated [25, 26]. Polymer films, e.g. polyamides, silicone elastomers or photoresists, are often used as structural material or masks and are deposited in a number of ways, e.g. through moulding, spin coating or CVD [37]. When a thin film is applied through spin coating, the rotational velocity and the spinning time decide the layer thickness. The use of thin films of biomolecules, generally proteins, is increasing. These can be deposited through one of three methods; protein absorption, photochemistry or self-assembling monolayers [38].

Thin films of silicon dioxide and photoresist were used in the work presented in this thesis. In both cases the films acted as masking layers during the fabrication process. The silicon dioxide film was thermally grown and the photoresist film was applied through spin coating.

2.3 Lithography

When a suitable substrate material has been chosen, the design is generally transferred to it through photolithography [25]. In the first step of this process, the design is transferred to a mask, a glass sheet with a thin film of an opaque material on one side (e.g. chromium) that has been covered by a light sensitive polymer, i.e. photoresist. The transfer is done using a pattern generator which exposes the photoresist according to the desired pattern with e.g. laser or UV light [25]. The exposed areas are then dissolved to expose the chromium. In the next step the exposed chromium is etched away. Thus, the pattern has been transferred to the mask. The quality of the mask often sets the baseline for the resolution of the features that can be fabricated. When the mask is ready, the substrate is spin coated with photoresist. After the photoresist has been left to dry, the mask is placed on top of it and it is exposed to UV light through the parts of the mask where the opaque material has been removed. When negative photoresist is used the polymer chains in the exposed parts are cross-linked to make it less dissolvable. Correspondingly, when positive photoresist is used the polymer chains are instead broken up to make it more dissolvable. Thus, when placed in a suitable solvent, exposed positive photoresist and unexposed negative photoresist is dissolved, thereby exposing the underlying substrate for further processing, e.g. etching. In silicon microfabrication, the pattern is often subsequently transferred to an underlying layer of silicon dioxide. This is done by etching through

the exposed silicon dioxide using buffered hydrofluoric acid. The remaining photoresist is dissolved.

Since basically the same process is used to transfer the pattern to masks and substrates, direct patterning of the substrates can also be utilized. However, since it only takes seconds to expose one substrate using a mask and UV light lithography and hours to do the same exposure using a pattern generator, the latter is only recommended when a single substrate is to be processed.

There are several alternatives to UV light lithography, e.g. electron beam, X-ray, ion beam and soft lithography [26, 39-42]. These methods are considerably more complex but offer advantages compared to more conventional methods, e.g. higher resolution.

A pattern generator utilizing laser light and chromium masks was used in the work presented in this thesis. The masks were then used to transfer patterns to photoresist films on silicon substrates covered by silicon dioxide through UV light lithography.

2.4 Etching

There are two main etching methods available for microfabrication, wet etching (liquid phase) and dry etching (gas/plasma phase) [26]. Wet etching is a purely chemical process that can be either isotropic, i.e. the etch rate is the same in all directions of the material, or anisotropic, i.e. not the same in all directions. The prior results in a round etch profile while the latter results in a profile that is determined by the crystal lattice structure of the substrate or the process used. A mixture of hydrofluoric acid and nitric acid in water or acetic acid is normally used for isotropic etching of silicon substrates while solutions of potassium hydroxide (KOH), tetramethylammonium hydroxide (TMAH) or ethylenediaminepyrocatechol (EDP) are used for anisotropic etching [18, 27]. When etching silicon with KOH {110}-planes are typically etched at a rate one order of magnitude faster than {111}-planes and {100}-planes are etched two orders of magnitude faster [26]. Wet etching is a relatively simple and low cost procedure that produces well defined structures with smooth surfaces. However, since the freedom of design often is limited by the structure of the crystal lattice, dry etching has become an attractive alternative, even though it is considerably more complex and expensive. Dry etching includes three processes; reactive ion etching (RIE), sputter etching and vapour phase etching [26, 29]. RIE is a chemical process that occurs when plasma composed of ions, electrons and neutrons in a fully or partially ionized gas is transported to the surface of a substrate where material is removed as reaction products. Sputter etching removes material when high energy ions collide with the substrate surface and knock out atoms. Vapour phase etching is a chemical process in which gases react with the surface of the substrate and material is removed as products of these reactions. The available dry etching techniques utilize one or more of these processes. A development of the RIE process, termed deep reactive ion etching (DRIE), has recently more or less revolutionized microfabrication [43]. Using DRIE it is possible to rapidly etch hundreds of micrometers deep trenches with vertical side walls. However, the surface smoothness is considerably poorer compared to wet etching.

In addition to silicon, other substrate materials, e.g. glass, silicon dioxide and plastics, can also be etched [18]. Glass is most commonly isotropically wet etched using hydrofluoric acid. Pure SiO_2 can be RIE etched while sputter etching is preferable for non-pure glass substrates. Plastics substrates are generally sputter etched.

In the work presented in this thesis, silicon substrates were anisotropically wet etched using KOH and thin films of silicon dioxide were etched using buffered and non-buffered hydrofluoric acid. DRIE of silicon substrates was also evaluated but proved to offer insufficient wall parallelity and surface smoothness compared to KOH wet etching.

2.5 Bonding

Microfabrication often requires hermetic sealing between two substrate surfaces. To achieve this there are a number of bonding methods available, some with and some without intermediate layers. The most widespread method for bonding of silicon substrates to borosilicate (Pyrex) substrates is anodic bonding, i.e. bonding assisted by an electrical field [27]. The silicon substrate is placed on a hot plate (350-450°C) and the borosilicate substrate is placed on top of it. A cathode is then attached to the top surface and a voltage is applied (preferably 400-700 V DC). At the elevated temperature the sodium ions in the glass are able to migrate towards the negative pole, leaving a negative charge in the region next to the silicon-glass interface. This results in an electrostatic pressure between the two substrates that pulls them together. At the elevated temperature covalent bonds are believed to form between the surfaces even though the exact nature of the process is still open for speculation. Two silicon surfaces can be bonded together using fusion bonding [27]. In this process two ultra clean surfaces are brought into contact at ~1000°C, whereby a perfect bond with no visible interface is formed. Although fusion bonding can be used for a wide variety of materials, it is most often used for silicon-silicon and silicon dioxide-silicon dioxide bonding. There are also additional methods for glass-glass bonding [44]. Next to the above mentioned methods there are several others for specific materials and applications. Some are based on adhesives and some on simple heating of the substrates, some are reversible and some are irreversible.

Anodic bonding of borosilicate glass to silicon was used in the work presented in this thesis.

2.6 Microfabrication of a microchannel

The microfabrication of the silicon and borosilicate glass flow channels used in the work presented in this thesis is summarized in figure 2.3. Initially, a silicon dioxide layer is deposited on top of a silicon substrate through thermal oxidation (1 and 2). The new substrate surface is spin coated with positive photoresist (3). Following UV light lithography the exposed photoresist is dissolved (4 and 5). The silicon dioxide not covered by photoresist is then etched away using buffered hydrofluoric acid (6). At this stage the remaining photoresist can be removed using acetone since the

pattern is defined by the silicon dioxide layer (7). In the next step, flow channels of different cross-section geometry are anisotropically wet etched using KOH, while the silicon dioxide layer acts as an etching mask (8). When the desired channel dimensions have been reached the remaining silicon dioxide is removed and the channels are sealed with borosilicate glass through anodic bonding (9 and 10).
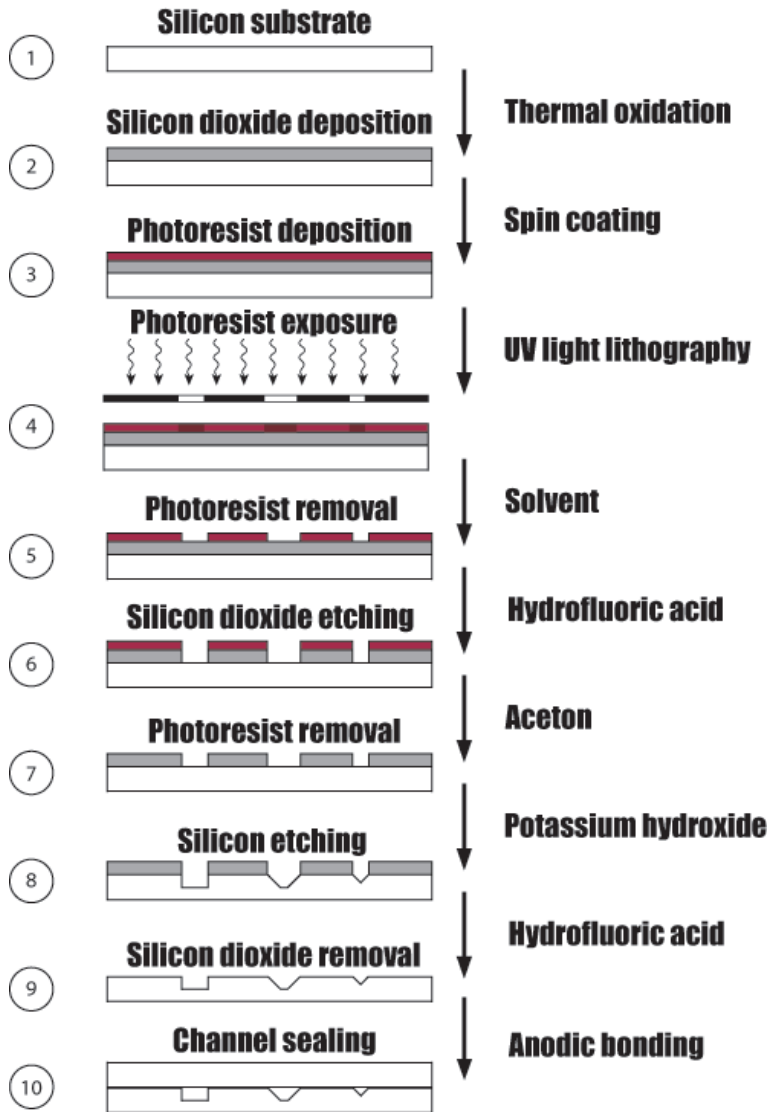

Figure 2.3: *The microfabrication process sequence of a silicon and borosilicate glass microchannel.*

3. Microfluidics

During the 1980s, when microfluidics was in its early stages of development, the field was dominated by microflow sensors, microvalves and micropumps [45]. This changed when its great potential in life science and chemistry became evident [12, 19]. Today, microfluidics is a highly interdisciplinary field that often combines e.g. physics, chemistry, engineering and biotechnology. Microfluidic devices are expected to play a major role in the near future, e.g. in medical diagnostics, molecular biology, genetic sequencing, chemical production, drug discovery and proteomics. It is possible that the impact one day will become comparable to that of microelectronics. There are already a wide variety of devices available and their applicability is steadily increasing [13, 46-50].

Fluidics in general and microfluidics in particular are complex research fields that involve many more or less well described phenomena. This chapter will give a general overview of fluidics with a focus on what is relevant in the context of this thesis. More extensive reviews on the subject can be found in the literature [11, 50-54].

3.1 Fluid mechanics

A fluid can be defined as any gas or liquid that cannot sustain a shearing force when at rest and that undergoes a continuous change in shape when subjected to such a stress [55]. Most water-like fluids can be treated as Newtonian fluids, i.e. fluids where the shear stress is linearly proportional to the velocity gradient in the direction perpendicular to the plane of shear. The constant of proportionality is the viscosity of the fluid, i.e. the fluids resistance to flowing (equation 3.1) [51].

$$\tau = \eta \cdot \frac{dv}{dx}$$
(eq. 3.1)

where τ is the shear stress, η is the dynamic (absolute) viscosity, and dv/dx is the velocity gradient perpendicular to the direction of shear.

When designing microfluidic devices it is important to be aware of the properties of the fluid as well as those of the flow. These properties can be organized in four groups; kinematic, transport, thermodynamic and miscellaneous [50]. Kinematic properties are typically velocity, acceleration and strain rate while transport properties refer to e.g. viscosity, thermal conductivity and diffusivity. Thermodynamic properties include pressure, temperature and density. Other properties, like surface tension, vapour pressure and surface accommodation coefficients, are categorized as miscellaneous. Starting out from these properties, calculations and simulations of the behaviour of fluid flows in various situations can be done. The simulations found in the literature are mainly based one out of two approaches, the molecular and the continuum [50]. The molecular approaches are generally based on the Lennard-Jones potential model. Two popular techniques are molecular dynamics (MD) and Direct Simulation Monte Carlo (DSMC). In the continuum approaches it is assumed that the

fluid can be treated as a continuum, i.e. all relevant properties are defined everywhere in space and vary continuously. Computational fluid dynamics (CFD) is the most popular technique. The approximate length scale above which the continuum approach can be expected to give a reliable result is ~1 μm for gases and ~10 nm for liquids [50].

3.1.1 Laminar flows

A fluid flow is described as laminar if it is smooth and follows predictable paths, streamlines. The word "laminar" refers to the fact that the fluid can be regarded as a number of indistinguishable laminated streams, laminae, flowing alongside each other. In applications of fluidics where mixing is undesirable it is important to make sure that the flow is kept laminar, i.e. not turbulent. Reynolds number, defined by equation 3.2, is a good laminarity predictor in long and straight flow channels with constant cross-section geometry [50].

$$\mathrm{Re} = \frac{\rho \cdot D_h \cdot u}{\eta} = \frac{D_h \cdot u}{v} \qquad \text{(eq. 3.2)}$$

where ρ is the density of the fluid, D_h is the hydraulic diameter (defined by equation 3.3), u is the characteristic velocity of the flow and v is the kinematic viscosity. The hydraulic diameter is an approximation technique that can be used both for completely and partially filled channels.

$$D_h = \frac{4 \cdot A}{P_{wet}} \qquad \text{(eq. 3.3)}$$

where A is the cross-section area of the flow and P_{wet} is the wetted perimeter, the perimeter of the channel that is in direct contact with the fluid.

Since D_h is small in the microscale domain, microfluidic flows are almost always laminar, a major advantage compared to macroscale flows. The transitional Reynolds number, above which a flow is expected to turn turbulent, generally ranges between 1000 and 2000, depending on factors like channel shape, aspect ratio and surface roughness [50].

Even though the advantages of laminar flows are many it is often desirable to mix fluids at specific points in microfluidic systems, e.g. when two reactants are to react efficiently. This seemingly trivial problem has proven to be a complex challenge. However, the efforts made to overcome it have resulted in a number of more or less efficient mixing methods [56].

3.1.2 Flow profiles

When a Newtonian fluid flows through a pipe under laminar conditions the velocity closest to the perimeter practically reaches zero due to friction, while it reaches its

maximum in the centre of the cross-section. The resulting rotary symmetrical velocity profile is described according to equation 3.4 [57].

$$v(r) = \frac{\Delta P}{4 \cdot \eta \cdot L} \cdot \left(R^2 - r^2\right)$$ (eq. 3.4)

where R is the radius of the pipe, r is the distance from the centre of the cross-section of the pipe, L is the length of the pipe and ΔP is the pressure difference between the beginning and the end of the pipe. As can be seen in equation 3.4 the flow profile has a parabolic shape, which is characteristic for laminar flows.

Microfluidic flow channels have rectangular rather than circular cross-sections. The velocity profile of a rectangular cross-section flow channel, described by equation 3.5, is considerably more complex [58]. The flow profile along the symmetry axes of the channel cross-section is typically parabolic in the shorter dimension of the cross-section while it gets blunter and blunter in the other as the aspect ratio increases.

$$v(x,z) = \frac{dP}{dy} \cdot \frac{1}{2 \cdot \mu} \cdot \left\{ \left[z(z-w)\right] + \sum_{m=1}^{\infty} \sin\left(\frac{m \cdot \pi \cdot z}{w}\right) \left[\begin{array}{l} A_m \cdot \cosh\left(\frac{m \cdot \pi \cdot x}{w}\right) + ... \\ ... + B_m \cdot \sinh\left(\frac{m \cdot \pi \cdot x}{w}\right) \end{array} \right] \right\}$$ (eq. 3.5)

$$A_m = \frac{-2 \cdot w^2}{m^3 \cdot \pi^3} \left[\cos(m \cdot \pi) - 1\right]$$ (eq. 3.6)

$$B_m = \frac{-A_m \left[\cosh\left(\frac{m \cdot \pi}{a_R}\right) - 1\right]}{\sinh\left(\frac{m \cdot \pi}{a_R}\right)}$$ (eq. 3.7)

$$a_R = \frac{w}{d}$$ (eq. 3.8)

where P is the pressure in the channel, d is the channel depth, w is the channel width, x is the channel depth coordinate, y is the channel length coordinate, z is the channel width coordinate and a_R is the aspect ratio of the channel.

Since few fluids are perfectly Newtonian and this affects the appearance of the flow profile it is advantageous to know how non-Newtonian fluids behave. There are two main classes, time-independent and time-dependent non-Newtonians [52]. Fluids sorting under the prior group have a given viscosity at a given shear stress that does not vary with time while those belonging to the latter group have a given viscosity at a given shear stress that varies with time. Time-independent non-Newtonian fluids are further subdivided into pseudoplastic (shear thinning, e.g. blood), dilatant (shear

thickening, e.g. starch in water) and plastic (solids until a certain shear stress is reached, e.g. toothpaste or grease). Time-dependent non-Newtonian fluids are subdivided into thixotropic (time-thinning, e.g. yoghurt) and rheopectic (time-thickening, e.g. gypsum paste).

3.1.3 Entrance effects

When a fluid flows from a macroscale vessel into a microchannel with constant cross-section geometry, a certain distance along the direction of flow is required for the laminar flow profile to become fully developed. When designing microfluidic systems, entrance effects should be considered. In low Reynolds number flows the entrance length can be approximated according to equation 3.9 [50].

$$\frac{L_e}{D_h} \approx \frac{0.6}{1 + 0.035 \cdot \text{Re}} + 0.056 \cdot \text{Re} \qquad \text{(eq. 3.9)}$$

where L_e is the entrance length and Re is the Reynolds number based on the hydraulic diameter D_h.

3.1.4 Driving flows

Pressure gradients are probably the most common way of driving flows in microfluidic systems. An under-pressure is applied to the outlets of the system or an over-pressure is applied to the inlets using e.g. syringe pumps or roller pumps. Integrated micropumps are also common [50]. In addition to pressure gradients, methods based on electric fields (e.g. electro-osmosis, electrophoresis and dielectrophoresis), acoustic streaming (e.g. quartz wind, boundary induced streaming and cavitation microstreaming), magnetic fields (e.g. magnetohydrodynamic stirring), capillary effects (e.g. surface tension and surface tension gradients) and fluid-structure interactions (e.g. transport through movement of the channel walls) can be utilized [53, 54].

3.2 Microparticles in fluids

Microparticles are common in current as well as potential future microfluidic applications. Suspended microparticles, e.g. organic and inorganic beads with or without surface coatings and cells, are routinely handled in e.g. separation, sorting, chemistry and analytical applications [15, 59, 60]. In processes where microparticles are not naturally present they are frequently introduced, e.g. to increase surface to volume ratio, act as catalysts or carry active surfaces.

It is desirable to keep track of particles inside microfluidic systems and to be able to characterize them before and after they have passed through the systems, mainly in order to optimize processes like e.g. mixing, pumping, separation and filtration. Straightforward ways to do this is to observe them through a microscope during system operation and to look at particle suspension samples on microscope slides

before and after. However, there are more efficient and informative methods available.

3.2.1 Particle size measurement

Particle size distribution measurements reveal information about the process the particles have been subjected to, e.g. throughput, damage done to the particles and how the particles have moved through the system. There are a number of methods available to perform these measurements [61]. However, it is important to bear in mind that particle size values always come in the form of equivalent diameters based on e.g. the projected perimeter diameter, the projected area diameter, the sphere of equivalent volume diameter or averages of such diameters. Two of the most popular methods are the electrical sensing zone (ESZ) and laser light scattering methods.

The ESZ method, also termed the Coulter Counter principle, is based on the measurement of impedance changes between two electrodes submerged in an electrolyte, e.g. sodium chloride solution. The electrodes are located on either side of a small aperture through which the sample suspension is sucked. As the particles pass through the aperture, one by one, the impedance between the electrodes changes momentarily since the electrolyte is displaced. These changes are converted into voltage pulses that are proportional to the volumes of the particles. The sphere of equivalent volume diameters are then calculated from these values.

While the ESZ method measures particles one at the time, the laser light scattering method measures groups of particles by illuminating them with laser light and recording the pattern created on the other side by the scattered and refracted light. The pattern is computer processed in order to extract information about the particle size distribution. The result comes in the form of the projected area diameters.

In their most modern forms the ESZ and laser light scattering methods are comparable in performance in liquid phase applications. Both methods are capable of measuring particles in a range from tenths of micrometers to thousands of micrometers.

3.2.2 Particle tracking

The possibility to track particles within a microfluidic system offers a great opportunity to understand the fluid dynamics within the system. Some particle tracking methods can be integrated while others require an optically transparent window into the area of interest.

The simplest form of particle tracking is to measure the flow velocity at a specific point, i.e. through pointwise methods [50]. To do this there are mainly two methods available, Laser Doppler Velocimetry (LDV) and Optical Doppler Tomography (ODT). However, full-field methods, that offer at least two-component velocity measurements within a two dimensional plane, are preferable [50]. The dominating full-field methods are Scalar Image Velocimetry (SIV), Molecular Tagging Velocimetry

(MTV) and Particle Image Velocimetry (PIV). The fact that SIV and MTV utilize molecular tracers makes them less useful in particle monitoring applications since particles can be affected by more than the flow. Consequently, PIV has become the method of choice in microfluidics [62-64]. The basic principle is very simple, moving suspended particles are photographed two or more times and the images are cross-correlated to determine how far the particles have moved. The information is then processed in order to generate e.g. particle trajectories and velocity vector fields.

4. Acoustics

Acoustics is the branch of physics that is dedicated to the studies of sound, i.e. the generation, transmission, control, reception and effects of mechanical waves in solids, liquids and gases [55]. There are several major sub-branches in this research field but the more prominent ones, in addition to ultrasonics, are environmental, architectural, musical and engineering acoustics. Ultrasonics is the study of sound with a frequency higher than audible sound, environmental acoustics deals with noise control, architectural acoustics is the study of how sound waves and buildings interact, musical acoustics deals with the design and use of musical instruments and how they affect the listener and engineering acoustics concerns the recording and reproduction of sound.

The first section of this chapter outlines the basics in acoustics with an emphasis on ultrasound and other relevant matters in the context of this thesis. Next, acoustic forces on suspended particles are described. Finally, acoustic streaming, a common phenomenon when microfluidics and sound waves are combined, is described. More detailed and extensive reviews on acoustics can be found in the literature [57, 65-69].

4.1 Sound

Sound propagates through a medium as longitudinal waves, i.e. compressions and rarefactions along the direction of propagation of the wave. Transverse waves, on the other hand, are oscillations perpendicular to the direction of propagation, e.g. waves on a string. Since longitudinal and transverse waves in most situations are governed by the same laws of physics and are described in the same way mathematically it is often convenient to think of sound waves as transverse waves.

4.1.1 Travelling waves

A simple sinusoidal sound wave travelling in the positive x-direction is described by the wave function (equation 4.1) [57]. The wave function satisfies the wave equation (equation 4.2), one of the most important equations in physics [57]. This signifies that the wave function describes a disturbance that can propagate along an axis at a specific wave speed (equations 4.3 and 4.4) [70]. Since liquids in general, contrary to solids, cannot support shear stress, only longitudinal waves can pass through them.

$$y(x,t) = A \cdot \sin(\omega t - kx) \tag{eq. 4.1}$$

$$\frac{\partial^2 y(x,t)}{\partial x^2} = \frac{1}{c^2} \cdot \frac{\partial^2 y(x,t)}{\partial t^2} \tag{eq. 4.2}$$

$$c = \sqrt{\frac{B}{\rho}} \qquad \text{(liquids, longitudinal wave)} \tag{eq. 4.3}$$

$$c = \sqrt{\frac{\left(B + \frac{4}{3}G\right)}{\rho}} = \sqrt{\frac{E(1-\nu)}{\rho(1-\nu-2\nu^2)}} \qquad \text{(solids, longitudinal wave)} \qquad \text{(eq. 4.4)}$$

where y is the displacement, x is the position, t is the time, A is the displacement amplitude, ω is the angular frequency (defined as $2\pi f$, where f is the frequency), k is the wave number (defined as $2\pi/\lambda$, where λ is the wavelength), c is the wave speed, B is the adiabatic bulk modulus, G is the shear modulus (modulus of rigidity), ρ is the density of the medium, E is the Young modulus and ν is the Poisson ratio.

Since sound waves propagate as compressions and rarefactions in a medium it is practical to express the wave function in gauge pressure form (equation 4.5) [57]. The gauge pressure is the pressure above or below atmospheric pressure. Note the fact that the pressure function is phase shifted 90° compared to the displacement function.

$$p(x,t) = p_0 \cdot \cos(\omega t - kx) \qquad \text{(eq. 4.5)}$$

$$p_0 = B \cdot k \cdot A \qquad \text{(eq. 4.6)}$$

where p is the gauge pressure and p_0 is the gauge pressure amplitude.

4.1.2 Standing waves

Interference between sound waves can generally be handled through superpositioning, i.e. by adding the displacement of all the wave functions in every point. A special case of wave interference is when two travelling waves of equal amplitude and wavelength meet head on. The result is a phenomenon known as a standing or stationary wave. A standing wave has displacement nodes, where the medium particles do not move, and displacement antinodes, where the particles move twice as much as in one of the travelling waves. Contrary to travelling waves, standing waves do not transfer energy, i.e. the net energy transport in both directions is zero. The displacement and pressure functions of a standing wave are given by equation 4.7 and 4.8 [57, 71].

$$y(x,t) = A_{SW} \cdot \cos(\omega t) \cdot \sin(kx) \qquad \text{(eq. 4.7)}$$

$$p(x,t) = -p_{SW} \cdot \cos(\omega t) \cdot \cos(kx) \qquad \text{(eq. 4.8)}$$

where A_{SW} and p_{SW} are the sums of the amplitudes of the individual waves.

The pressure and displacement functions are 90° out of phase with respect to position, but not with respect to time. Thus, pressure nodes will always be displacement antinodes and pressure antinodes will always be displacement nodes.

4.1.3 Ultrasound

Depending on the frequency of the sound waves, sound is referred to as infrasound (f < 20 Hz), audible sound (20 Hz ≤ f ≤ 20 000 Hz) or ultrasound (f > 20 000 Hz). Two of the main application areas of ultrasound are in medicine, where it is used e.g. in diagnostic and therapeutic applications, and in the industry, where it is used e.g. in material defect detection, cleaning and welding applications [68, 72-75]. Other areas of application can be found in e.g. chemistry, biology, communication and warfare [65, 76-78].

Sound waves are generated or received by transducers. Reversible transducers can be used in both roles [70]. There are several transducer types available but high frequency ultrasonic waves in solids and liquids are most often generated and received by piezoelectric transducers. The functionality of these is based on a phenomenon known as the piezoelectric effect. This effect converts electrical oscillations into mechanical vibrations and vice versa. Nowadays, piezoelectric ceramics, e.g. barium titanate and lead zirconate titanate, are commonly used fabrication materials. Before these materials were made available, transducers were made from quartz crystals or ferromagnetic materials [65, 70]. In the latter case the magnetostrictive effect was utilized [70].

A piezoelectric ceramic is a polycrystalline material composed of a mass of minute crystallites with one positively and one negatively charged part [79]. When a ceramic plate, with electrodes located on its opposing faces, is heated to a temperature close to its Curie temperature under the influence of a strong electrical field, some of the crystallites align such that their negatively charged ends point towards the positive electrode. During this process a dimensional change takes place as a result of the shape of the crystallites. When the ceramic is cooled and the field is removed this alignment is made permanent. Thus, the ceramic plate is polarized and will stay so until it is heated to its Curie temperature, at which the polarization is lost. When a voltage is applied to the electrodes of the polarized ceramic it will either lengthen or shorten depending on the polarity of the voltage. This happens because more crystallites are aligned in the same direction as the permanently aligned ones or the permanently aligned ones temporarily loose their alignment. If an altering voltage is applied, the plate will oscillate at the frequency of the voltage and send out vibrations, sound waves, in the surrounding medium.

4.1.4 Attenuation of sound waves

When sound waves propagate through a medium they are attenuated, i.e. the intensity in the direction of propagation is reduced. Attenuation is caused by two processes, absorption of the sound waves and deviation of energy from the direction of propagation [70]. Through absorption, some of the mechanical energy of the waves is converted into heat by internal friction. Deviation of energy from the direction of propagation is caused by reflection, refraction, diffraction and scattering. In addition to the medium itself, suspended particles also contribute to the attenuation [80].

Beers-Lambert-Bouguer law, equation 4.9, describes the attenuation process and also defines the frequency dependent absorption coefficient [65, 70, 72].

$$I = I_0 \exp(-2\alpha x) \qquad\qquad \text{(eq. 4.9)}$$

where I is the intensity of the wave after it has travelled the distance x through the medium, I_0 is the intensity at x = 0 and α is the absorption coefficient.

In fluids, there are two main causes of absorption, viscosity and thermal conduction [70]. The viscous losses can be regarded as friction losses between the molecules of the medium as they move relative to each other during the propagation of the sound waves through the medium. Thermal conduction losses arise during heat transport between the warmer compressed regions of the medium and the cooler rarefied regions. Relaxation phenomena can also affect the absorption characteristics but that is beyond the scope of this thesis.

Compared to fluids, there are more potential causes of attenuation of sound waves in solids [70]. These are characteristic of the physical properties of the medium and can be categorized in a number of groups; losses characteristic of polycrystalline solids, absorption due to lattice imperfections, absorption in ferromagnetic and ferroelectric materials, absorption due to electron-photon interactions, absorption in single crystals due to thermal effects and absorption due to other possible causes, e.g. acoustoelectric effects, structural relaxation, thermal relaxation and nuclear magnetic resonance. The details of these processes are beyond the scope of this thesis.

4.2 Acoustic force theory

It is easy to picture that suspended particles are affected by forces when sound waves propagate through their suspending medium. The waves are disturbances in the positions of the medium particles and these consequently exert forces on both their neighbouring medium particles and the suspended particles as they move. However, in most cases these forces are very small or cancel each other out. Though, in some particular situations, e.g. in standing waves, this is not always the case. Under certain conditions, acoustic standing wave forces make suspended particles move in a controlled fashion [81-87]. The involved acoustic forces can be divided into two categories; the primary acoustic radiation forces (PRFs), generated by the interaction of a particle with the primary wave field, and the secondary acoustic radiation force (SRF), generated by the interaction between a particle and the scattered wave field [88-90].

4.2.1 Primary acoustic radiation forces

The investigation of acoustic radiation forces began in 1874 when Kundt and Lehmann demonstrated that suspended particles can be affected by forces generated in an acoustic standing wave [1]. Nevertheless, the theoretical breakthrough came more than half a century later, in 1934, when King derived an expression for the

radiation pressure on a rigid sphere, much smaller than the wavelength of the sound waves, suspended in a non-viscous fluid [83]. This work was extended to include compressible spheres by Yosioka and Kawasima in 1955 and, using another approach, Gor'kov confirmed their results in 1962 [82, 84]. A complementary approach to the understanding of the acoustic radiation force, based on kinetic and potential energies, was presented by Nyborg in 1967 [91]. However, with small variations, it is in the Gor'kov, Yosioka and Kawasima form we are used to see the acoustic force equation, equation 4.10 [89, 90, 92-96]. To be more precise, this equation describes the axial PRF, F_{ax}, which is the force responsible for moving suspended particles to the nodal and anti-nodal planes of a standing wave. In most acoustic particle manipulation applications this is the most relevant acoustic force.

$$F_{ax} = -\left(\frac{\pi p_0^2 V_p \beta_m}{2\lambda} \right) \cdot \phi(\beta, \rho) \cdot \sin(2kx) \qquad \text{(eq. 4.10)}$$

$$\phi(\beta, \rho) = \frac{5\rho_p - 2\rho_m}{2\rho_p + \rho_m} - \frac{\beta_p}{\beta_m} \qquad \text{(eq. 4.11)}$$

where the densities of the medium and the particles are denoted ρ_m and ρ_p, respectively, and the corresponding compressibilities β_m and β_p, respectively, V_p is the volume of the particle, ϕ is the acoustic contrast factor or the ϕ-factor, defined by equation 4.11, and x is the distance from a pressure node. The compressibility is the reciprocal of the bulk modulus and can e.g. be determined through acoustic methods [57, 97].

The direction of the axial primary acoustic radiation force is determined by the sign of the ϕ-factor, a positive ϕ-factor results in movement towards a pressure node and a negative ϕ-factor results in movement towards a pressure anti-node correspondingly (figures 4.1 and 4.2).
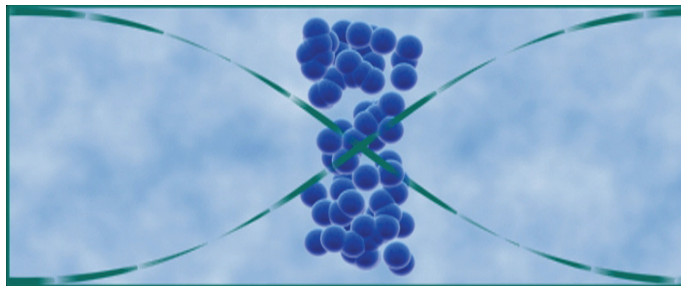

Figure 4.1: *Positive ϕ-factor particles are moved to the pressure nodal plane of the standing wave under the influence of the axial PRF.*
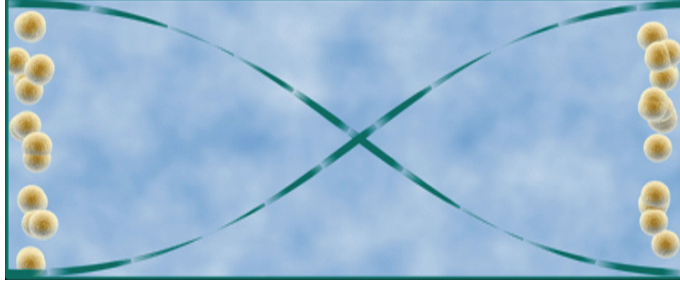
Figure 4.2: *Negative ϕ-factor particles are moved to the pressure anti-nodal planes of the standing wave under the influence of the axial PRF.*

In addition to the axial PRF there is a transverse PRF, equation 4.12, acting in the plane perpendicular to the direction of the standing wave [80, 90, 95, 98, 99]. The transverse PRF has been found to aggregate particles at the local acoustic energy density maxima of the pressure nodal planes [90]. These maxima can be caused by e.g. a non-uniform amplitude distribution of the source, divergence of the wave and the influence of the boundaries [80].

$$F_{tr} = V_p \nabla E_{ac} \left[\left(\frac{3(\rho_p - \rho_m)}{\rho_m + 2\rho_p} \right) \cos^2(kx) - \left(\frac{\beta_m - \beta_p}{\beta_m} \right) \sin^2(kx) \right]$$ (eq. 4.12)

where ∇ is the transverse gradient operator and E_{ac} is the time-averaged acoustic energy density of the field.

4.2.2 Secondary acoustic radiation force

When two suspended particles are present in the same sound field, each particle will be subjected both to the incident sound waves and the scattered waves from the other particle. The scattered waves give rise to an interaction force between the particles, the SRF. Pioneers in the investigation of this force were König and Bjerknes, which is why it is sometimes referred to as the König or Bjerknes force [100, 101]. Since then several researchers have considered the SRF [80, 88-90, 102-107]. Under the assumptions that the particles and the distance between them are much smaller than the wavelength of the sound waves, the SRF between two identical compressible spheres in a plane standing wave is described by equation 4.13 [80, 88].

$$F_{sr} = 4\pi a^6 \left[\frac{(\rho_p - \rho_m)^2 (3\cos^2\theta - 1)}{6\rho_m d^4} v^2(x) - \frac{\omega^2 \rho_m (\beta_p - \beta_m)^2}{9d^2} p^2(x) \right]$$
(eq. 4.13)

where a is the radius of the particles, d is the centre to centre distance between them, θ is the angle between the centreline of the particles and the direction of propagation of the incident acoustic wave and v(x) and p(x) are the velocity and pressure, respectively, of the unperturbed incident field at the position of the particles.

22

The sign of the force is to be interpreted such that a negative sign means that there is an attractive force between the particles and a positive sign means that there is a repulsive force. The first term of the right side of the equation depends on the particle velocity and the orientation of the particles with respect to the incident sound waves, while the second term depends on the acoustic pressure. When particles are lined up in the direction of the wave propagation ($\theta = 0°$) the first term is repulsive and when the centreline of the particles is perpendicular to the direction of the wave propagation ($\theta = 90°$) it is attractive. The second term is always attractive. Note that the first term vanishes at the velocity nodes and the second term vanishes at the pressure nodes. The influence of the SRF is small compared to the axial PRF. The maximum SRF is about two orders of magnitude weaker than the maximum axial PRF, the latter typically in the order of 10^{-11} N in acoustic particle manipulation applications [90]. Furthermore, the SRF is significant only when the particles are very close together. However, it can become important in e.g. aggregation and sedimentation applications, where particles are first brought together by the PRFs [88, 94, 108, 109].

In addition to the PRFs and the SRF, gas bubbles oscillating under the influence of an acoustic standing wave field can sometimes induce forces on suspended particles, provided that they are in very close proximity [89].

4.3 Acoustic streaming

In addition to acoustic forces on suspended particles, sound waves induce acoustic streaming. The study of these phenomena was pioneered by Lord Rayleigh [110, 111]. Nowadays, steady acoustic streaming phenomena are generally referred to one of three categories; Rayleigh streaming (boundary induces streaming), the Quartz wind or cavitation microstreaming [54, 112, 113]. Rayleigh streaming occurs around solid boundaries and is induced by standing waves between plane walls. This phenomenon results in rotating vortex flows and can be used in e.g. mixing applications [114]. The Quartz wind arises when a source projects a high intensity beam of sound into a body of fluid [115]. It was initially associated with quartz crystal oscillators generating ultrasound, hence the name. Both Rayleigh streaming and the Quartz wind owe their origin to the action of Reynolds stresses [116]. The third class of steady acoustic streaming, cavitation microstreaming, is induced through the interaction between bubbles and acoustic waves [117]. The streaming comes about when the centre of a bubble, which is attached to solid wall, moves away from the wall as the bubble expands. Cavitation microstreaming can be used in e.g. mixing applications [54].

5 Separation technology

Separation processes are undoubtedly among the more central phenomena in nature [118]. By looking at e.g. the structure of the earth, with its core, mantle, crust, hydrosphere and atmosphere, it is obvious that separation processes of gigantic proportions have been involved. In the other end of the size scale, processes inside the body deal with separation of cells, proteins, molecules and atoms in order to uphold life itself. Thousands of years ago, mankind learned to master simple separation processes, e.g. to extract components from minerals, plants or animals. In our time, complex separation processes are routinely used in laboratories and industries everywhere and the annual turnover of this market is in the order of billions of euros or more. In separation science, separations of suspended particles from their medium or from other particles are among the more common tasks. The particles involved are e.g. cells, subcellular fragments, molecular aggregates, molecules, crystals, bacteria, viruses or beads of various types.

In this chapter several common separation methods for suspended particles are briefly reviewed. These are often described and named after the underlying force, phenomenon or form of operation. Some are continuous while others are batch processes. Some can only handle relatively large particles while others only handle very small ones. In addition to three major groups of methods, based on magnetic, electrical and centrifugal/gravitational forces, several other approaches are discussed.

5.1 Magnetic methods

By applying an external magnetic field it is possible to move magnetic particles while in suspension [13, 119-123]. The basic concept is termed magnetophoresis and is a batch process in its simplest form. To separate the particles from their medium, a magnet is applied to the outside of the container. Magnetic particles, with or without other particles trapped on their surface, subsequently move to the wall closest to the magnet and remain there as the suspending medium, together with any non-magnetic particles, is decanted. This is followed by detachment and washing steps. Magnetic beads with an iron oxide core, a polymer shell and e.g. an antibody coating to which e.g. cells and biomolecules attach are often used [124]. There are also two naturally occurring magnetic cell types, erythrocytes (red blood cells) and magnetotactic bacteria [119]. Furthermore, some non-magnetic bioparticles can be made magnetic through absorption of magnetic materials [120, 125]. Free flow magnetophoresis is magnetophoresis in a continuous laminar flow mode [124]. Magnetic particles are deflected, perpendicular to the direction of flow, by a magnetic field and exit the separation zone through different outlets depending on how much they have been deflected. In addition to separation from non-magnetic particles, free flow magnetophoresis offers separation based on size and magnetic susceptibility, the latter a quantity describing the magnetic response of a substance to an applied magnetic field. Size base separation is possible since the size dependent drag force opposes the magnetic force. The drag force, equation 5.1, results from the friction between the medium and the particle (friction drag) and the displacement of the medium by the particle (form drag) [120]. The magnetic force exerted on a particle is given by

equation 5.4 [120]. Next to throughput, a drawback associated with free flow magnetophoresis is varying reproducibility of particle deflection [124].

$$F_d = \frac{1}{2} \cdot \rho_m \cdot v^2 \cdot C_D \cdot A \qquad \text{(eq. 5.1)}$$

$$C_D = \frac{24}{\text{Re}} \qquad \text{(for spherical particles and Re < 0.1)} \qquad \text{(eq. 5.2)}$$

$$C_D = \left(\sqrt{\frac{24}{\text{Re}}} + 0.5407 \right)^2 \qquad \text{(for spherical particles and 0.5<Re < 6000)} \qquad \text{(eq. 5.3)}$$

where ρ_m is the density of the medium, v is the particle velocity relative to the medium, C_D is the drag coefficient, A is the reference area (approximately the projected cross-section area of the particle perpendicular to the direction of its movement) and Re is the Reynolds number. Equation 5.2 can also be used for 0.1<Re<1 but this results in a slight underestimation of the drag force.

$$F_m = \chi \cdot H \cdot V_p \cdot \frac{dB}{dx} \qquad \text{(eq. 5.4)}$$

where χ is the magnetic susceptibility, H is the magnetic field intensity, V_p is the particle volume and dB/dx is the magnetic field gradient. The typical maximum magnetic field gradient is $\sim 2.5 \cdot 10^5$ T/m [120].

5.2 Electrical methods

Electrophoresis and dielectrophoresis are two groups of separation methods that are based on electrical fields [13, 50, 118, 120, 126, 127]. Electrophoresis, the movement of charged particles under the influence of an electrical field, is often combined with interaction between particles and surfaces, a variation referred to as electrochromatography [128-130]. The process starts as the sample suspension is placed in one end of an electrolyte or porous medium, on a plate or in a capillary. Next, an electrical field is applied in the desired direction of movement. The charged particles then start to move in the direction of the field at a rate determined by their charge, the field strength, the drag force and possible interactions with the solid phase. Particles that move faster are separated from particles that move slower. Basic electrophoresis is a batch process but there is a continuous version available, termed free flow electrophoresis [131-134]. This method is analogous to free flow magnetophoresis, with the difference that the particles move under the influence of an electrical field instead of a magnetic. The magnitude of the electrical force on a charged particle is given by equation 5.5 [120].

$$F_e = q \cdot E = 2 \cdot \pi \cdot d_p \cdot \left(1 + \frac{1}{2} \cdot \kappa \cdot d_p\right) \cdot \varepsilon \cdot \zeta_p \cdot E \qquad \text{(eq. 5.5)}$$

where q is the particle charge, E is the electrical field strength, d_p is the particle diameter, κ is the reciprocal of the Debye decay length, ε is the dielectric permittivity of the fluid and ζ_p is the zeta-potential of the particle.

The zeta-potential refers to the electrostatic potential generated by the accumulation of ions at the surface of the particle while the Debye decay length is a measure of the thickness of the electrical double layer around the particle [120]. The typical maximum electrical field strength in biological systems is ~$6 \cdot 10^6$ V/m [120]. Strong electrical fields often cause heating that can lead to unwanted convection.

When dielectric particles are exposed to an external electrical field they are polarized and form dipoles. If the field is inhomogeneous these dipoles experience a force and start to move. Depending on whether the movement is towards higher or lower field strength the phenomenon is referred to as positive or negative dielectrophoresis, respectively [135-138]. In this fashion, particles, e.g. cells, biomolecules or beads, can be trapped at local field strength maxima or minima, and are thereby separated from the suspending medium and non-dielectric particles.

5.3 Sedimentation methods

Suspended particles can be separated from each other or from their suspending medium under the influence of the gravitational or centrifugal force, i.e. through sedimentation (equation 5.6) [57, 118, 120, 139, 140]. In both cases, particles with a density that differs from that of the surrounding medium are forced through it, against the drag force. Separation through sedimentation is based on differences in density, volume and drag force.

$$F_c = a \cdot \Delta\rho \cdot V_p \qquad \text{(eq. 5.6)}$$

where a is the centrifugal acceleration and $\Delta\rho$ is the density difference between the particle and the suspending medium. The upper centrifugal acceleration limit is ~20 000g [120].

Gravitational sedimentation can be utilized when particles are heavy enough to sink to the bottom of their container relatively fast. However, in order to separate e.g. cells, subcellular fragments and biomolecules, centrifugal sedimentation is generally needed. When the suspension is rotated, particles denser than the medium migrate towards the outer wall of the centrifuge while particles lighter than the medium move towards its centre. However, since small and random differences in medium density can cause convection during centrifugation, a medium density gradient is often introduced such that the density increases along the radius of the centrifuge, from the centre and out [118]. There is a wide variety of density changing media available [139]. In most cases, sedimentation methods are batch processes but there are continuous versions available for some specific applications [141-143].

5.4 Other methods

In addition to the magnetic, electrical and centrifugal/gravitational force based methods there is a wide variety of other methods and families of methods available [13, 118, 120, 144]. Some are already of great importance in specific applications while others might be the separation methods of the future. However, many are primarily used for separation of molecular or macromolecular particles. In addition, they often have low throughput or limited applicability.

A straightforward and very commonly used separation method is filtration, which can be done in several consecutive steps [145, 146]. The separation is only based on size and accumulation of particles in the filter is inevitable, even though the latter can be reduced by applying a flow tangential to the filter surface. Chromatography is also one of the more commonly used families of methods [118, 147]. In its simplest form, suspended particles travel through a column with a stationary phase, e.g. a tightly packed granular material, and separation of different particle types is achieved by differences in interaction with the stationary phase. Another family of methods, field-flow fractionation (FFF), separates suspended particles by combining a parabolic laminar flow profile with a perpendicular field or gradient in a thin flow channel [144, 148-153]. Dissimilar particles entering the channel in the centre of the flow profile or close to a wall follow different paths under the influence of the force field, while travelling through the channel. They subsequently exit the channel in sequence because of differences in mean flow rate during the pass. Any field or gradient that results in a desirable motion of the particles can be used. Split-flow thin fractionation (SPLITT) is a continuous form of FFF where a flow splitter at the end of the channel separates particles that have moved far enough to reach the other side of it from those who have not [148, 154]. Lately, deterministic hydrodynamic methods based on the hydrodynamic size of suspended particles in a laminar flow have been presented [155-157]. Arrays of flow splitters, where each row is slightly shifted in a direction perpendicular to the direction of flow compared to the prior, are used to achieve separation. The throughput is, however, extremely low which is also the case with a closely related method termed pinch flow fractionation [158-160]. By drastically reducing the cross-section of a laminar flow channel, large suspended particles are pushed from their original flow lines by the channel wall while smaller particles are able to remain. When the channel is widened again the particles can be separated by flow splitters since they follow different flow lines. Suspended particles can also be trapped using immunoaffinity methods that rely on biological differences rather than physical [161, 162]. Antibodies coupled to a solid phase catch particles that are able to bind to them. Furthermore, optical methods can be used to trap and separate dielectric particles in a continuous flow [13, 163].

This list could be made much longer, especially when it comes to molecular separation methods [13, 118, 120, 123, 145, 148, 164-168]. However, methods based on acoustic particle manipulation has been intentionally left out this far since it is the central topic of this thesis and therefore thoroughly described in chapters 6 & 7.

6. Acoustic particle manipulation

Acoustic forces induced by standing waves can be utilized to move or trap suspended particles. Most commonly, a standing wave is generated between two flat surfaces in a cavity. Suspended positive ϕ-factor particles in the cavity are subsequently moved to the pressure nodal planes of the standing wave under the influence of the axial PRF. Analogously, negative ϕ-factor particles are moved to the pressure anti-nodal planes. If the suspension is flowing under laminar conditions the flow profile can be split up by flow dividers such that the particles are separated from each other or from parts of the suspending medium. Alternatively, the increased local density of particles in the nodal and anti-nodal planes can be used to accelerate sedimentation. These basic concepts, in more or less modified forms, are employed to solve a variety of particle manipulation tasks. Consequently, a large number of scientific publications on the subject have been presented.

All particles, biological or non-biological, within a certain size range can be affected by acoustic forces provided that they have non-zero ϕ-factors. The upper size limit is set by the fact that the particles must have dimensions much smaller than the wavelength of the ultrasound used [83]. The lower size limit has not yet been explored in detail but it is likely that the weak acoustic forces on sub micrometer particles will be challenged by e.g. forces caused by streaming phenomena. On the other hand, the influence of other forces may very well offer new possibilities that can be taken advantage of. Although far from proven, it can be hypothesized that acoustic methods may very well offer a viable way forward in the domain of nanoparticle manipulation, i.e. manipulation of particles smaller than 1 micrometer.

In the first sections of this chapter the design of ultrasonic resonators and the modelling of the particle motion within them are reviewed. Next, a number of devices are discussed. Finally, the important question of viability of bioparticles in standing wave fields is considered.

6.1 Resonator design

The ultrasonic standing waves used in acoustic manipulation of suspended particles are often generated either by two opposing sound sources or, very commonly, by a single source facing a reflector. These two resonator design alternatives, where the standing wave is generated in the direction normal to the surface of the transducer or transducers, are referred to as layered resonators (figure 6.1). The sources used are generally piezoelectric ceramics that are coupled to a fluid layer, either directly or via a coupling layer. Quarter wavelength coupling layers are commonly used but the most favourable thickness depends on the characteristic acoustic impedances ($Z = \rho \cdot c$, where ρ is the density and c is the longitudinal wave speed) of the layers involved and the application. Combining a transducer, a quarter wavelength coupling layer, a half wavelength fluid layer and a quarter wavelength reflector generally results in a device capable of moving positive ϕ-factor particles to the pressure nodal plane in the middle

of the fluid layer and negative ϕ-factor particles to the pressure anti-nodal planes close to the coupling and reflector layer surfaces.
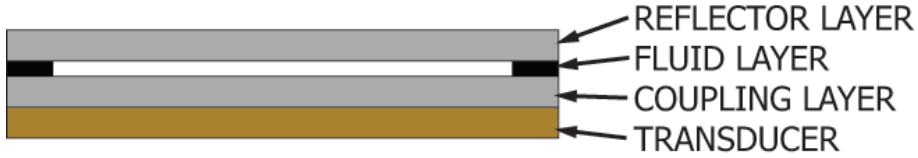

Figure 6.1: *Schematic illustration of the cross-section of a typical layered resonator, perpendicular to the direction of flow through the fluid layer. The centre part of the fluid layer is fluid filled while the outer part is a solid material that defines the thickness.*

Designing an ideal layered resonator with a specific resonance frequency has proven to be a difficult task. The choice of layer thicknesses and materials is a complex matter and much care must be taken in assembling the parts since these devices are very sensitive to misalignments. However, simulations of the response to changes in design parameters can give valuable design input. Important contributions in modelling and simulation of layered resonators have been made by a number of researchers. Gröschl described and extended mathematical modelling of layered resonators, from the electrical properties of the piezoelectric actuator to the generated acoustic field quantities in the fluid layer [80]. The model used was developed by Nowotny, Benes and Schmid and was experimentally validated by Hawkes, Gröschl and co-workers [80, 169-172]. Hawkes et al. used the model to explore a number of different resonator configurations [169]. Various combinations of half and quarter wavelength transmission, fluid and reflector layers were investigated in order to describe the pressure and acoustic energy density distributions. Further modelling by Hill and co-workers predicted that it may be possible to place a pressure nodal plane at an arbitrary position in the fluid layer of a resonator and that a transducer/transmission layer resonance very close to the fluid layer resonance should be avoided since the resonator becomes very sensitive to changes in geometric parameters [173-175].

A term often used when discussing resonators is the quality factor of the system (the mechanical Q-factor), i.e. the centre resonance frequency divided by the width of the range of frequencies for which the velocity amplitude is at least the peak value divided by the square root of two [70].

In addition to the layered resonators there are a number of less common resonator designs, e.g. cylindrical, two dimensional rectangular, hemispherical and confocal resonators [176]. A new type of resonator, developed by members of the nanobiotechnology and lab-on-a-chip group at the Department of Electrical Measurements at the Lund Institute of Technology, offers a simple and stable particle manipulation device, which is able to exploit the advantages of the microscale domain and is suitable for on-chip integration (figure 6.2) [177, 178]. The basic idea of the Lund design is to fabricate a resonance cavity in a piece of bulk material using microfabrication processes. The channel is then sealed and perfused with a particle suspension. As the bulk material is actuated by a piezoelectric ceramic attached to the back side of the bulk material, a standing wave is formed between the side walls of the channel if the actuation frequency corresponded to one of its harmonics.
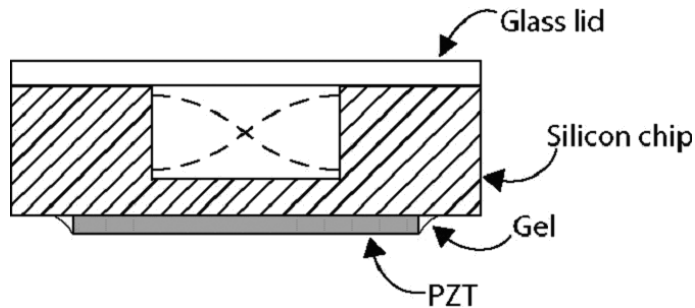
Figure 6.2: *Schematic cross-section of a separation chip utilizing the Lund method, perpendicular to the direction of flow through the channel. The channel was sealed by a borosilicate glass lid and was actuated from below using a piezoelectric ceramic. Ultrasonic gel was used to couple the transducer to the chip.*

Since the standing wave in the Lund resonator design is formed in a direction orthogonal to the direction normal to the transducer surface, a vibration mode conversion is likely to have occurred. This is presumed to be a result of the coupling between the oscillations in the two directions, i.e. when an object is compressed in one direction it is expanded in the other. This coupling can be illustrated using the Schlieren method [179]. Figure 6.3 shows that when a rectangular piece of brass is actuated by a piezoelectric transducer, sound waves are irradiated from it, both in the direction normal to the transducer surface and in the orthogonal direction. However, in order to reach the full potential of the Lund method the nature of the resonance used should be studied in more detail. In that context, the resonance behaviour in the direction normal to the transducer surface most likely needs to be examined more closely. Unfortunately, the possibility to evaluate different layer thicknesses is limited by the availability of substrate materials. The potential influence of the crystal lattice structure of silicon should also be considered.
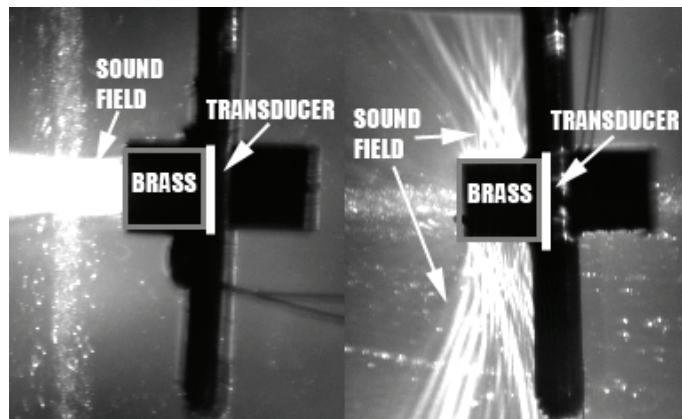

Figure 6.3: *The left part of the photograph, acquired using the Schlieren method, shows the sound field irradiated from a rectangular piece of brass (outlined) in the direction normal to the surface of the air backed transducer behind it. The right part of the photograph shows the sound fields in the perpendicular direction at the same moment.*

30

Though, without further speculations concerning how the sound waves find their way from the actuator to the channel, if one component in a physical system has a specific resonance frequency and the whole system is actuated at that frequency a resonance generally takes place in that component. It should also be noted that it is highly unlikely that the standing wave is ideal. For example, resonances in the length direction of the channel, variations in the near field of the transducer and disturbances from the surfaces inside the channel can be expected to distort the standing wave. In addition, in the equation describing the axial PRF (equation 4.10), it is assumed that the particles are not close to a wall, which they generally are in acoustic particle manipulation systems [180]. However, in most practical situations it is sufficient to picture the situation in the channel as an ideal standing wave in which the particles behave approximately as described by equation 4.10.

When designing an acoustic resonator the choice of construction materials is important. First of all, they must transfer sound waves efficiently in order to minimize heating of the device, i.e. materials with low acoustic attenuation are preferable. Secondly, in order to uphold a strong standing wave in the fluid layer, the layers in contact with it should display good acoustic reflection properties, i.e. the difference in acoustic impedance between the materials in contact with the fluid and the fluid should be relatively high. Lastly, there must be fabrication methods available for the specific materials in order to realize the resonator designs. Of course, compromises have to be made between these demands. Different types of glass or metals are suitable for transmission and reflector layers in the layered resonator design while silicon is suitable for the bulk material in the Lund design and borosilicate glass is suitable for sealing the channels. Unpublished results from the nanobiotechnology and lab-on-a-chip group in Lund have shown that it is possible to use various metals and even some plastic materials as alternatives to silicon but the latter is the most favourable material evaluated this far, mainly because of the fabrication methods available, the low attenuation and possibly because of the crystal lattice structure.

When actuating a resonator channel of the Lund design at its fundamental resonance frequency, positive ϕ-factor particles move to the single pressure-nodal plane in the middle of the channel, thereby forming a confined streaming band of particles with particle free medium on both sides. Consequently, negative ϕ-factor particles move to the pressure-anti-nodal planes close to the side walls. By adding flow splitters to form three outlets channels from the main channel, the particle stream can be collected in the centre outlet (positive ϕ-factor particles) or in the two side outlets (negative ϕ-factor particles) and the particle free fluid in the complementary outlet or outlets. Anisotropically wet etched silicon offers several options in designing the flow splitters. The most straightforward approach is to etch the two side outlet channels at a 90° angle in relation to the main channel (figure 6.4). A more favourable design, with respect to fluid dynamics, is one where the side channels are etched at a 45° angle in relation to the main channel (figure 6.5). Yet another design, with the flow splitters placed directly in the main channel, is possible if {110} oriented silicon is used instead of {100} oriented (figure 6.6). This offers a more chip area conservative solution in high throughput applications and when integrating the separation device with other microdevices.
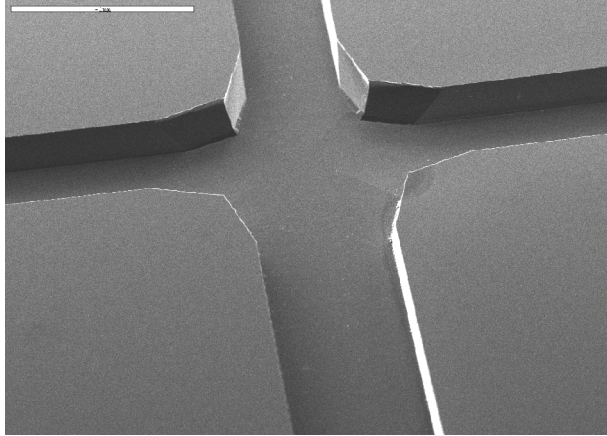
Figure 6.4: 90° design of flow splitters wet etched in a {100} oriented silicon wafer.
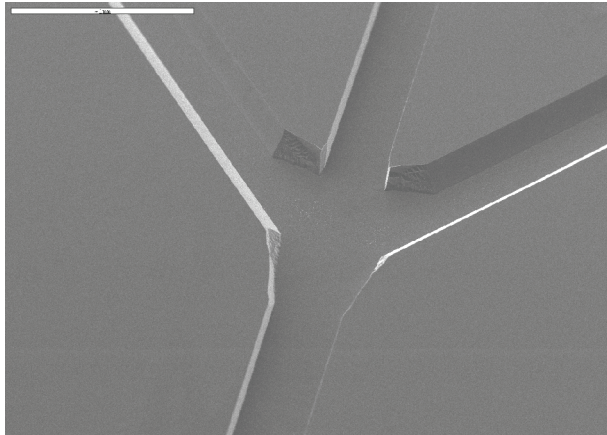

Figure 6.5: 45° design of flow splitters wet etched in a {100} oriented silicon wafer.
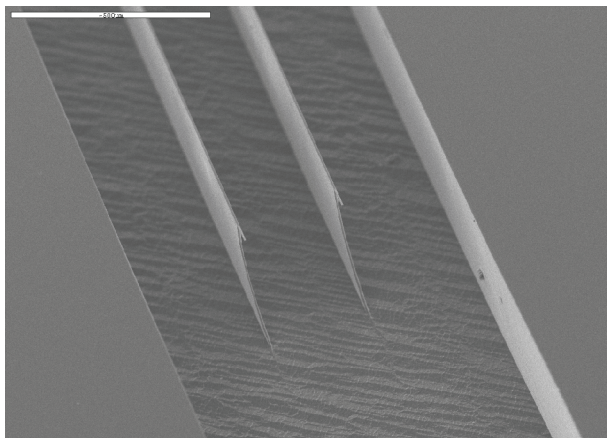

Figure 6.6: 0° design of flow splitters wet etched in a {110} oriented silicon wafer.

6.2 Particle movement models

The movement of suspended particles in acoustic particle manipulation devices has been investigated and described by several researchers [89, 181-186]. The resulting models can be used to predict how particles move in the devices but also to determine specific parameters based on the observed particle behaviour. In most cases, the relevant forces governing the movement of the particles are the axial PRF, the drag forces and in some cases gravity. If the suspended particles are flowing through a channel while being acoustically manipulated there are two drag forces involved, one caused by the fluid flow and one caused by the movement of the particle under the influence of the acoustic force. The influence of gravity is usually small but can be relevant in some devices. Depending on the density of the particles in relation to the suspending medium they will be negatively, neutrally or positively buoyant under the influence of gravity. There are of cause additional forces influencing the movement of particles in acoustic particle manipulation devices, e.g. the transverse PRF, interparticle forces, lift, wall interactions and forces induced by acoustic streaming, but these are often marginal compared to the other three.

6.3 Acoustic particle manipulation devices

Several researchers and groups of researchers have designed and built devices utilizing acoustic forces to manipulate suspended particles. Most of these devices are macroscale systems but in recent years microfabricated systems with the potential of being integrated with other microdevices have begun to appear. Presently, the number of such devices is small but the advantages associated with them are many. The following sections give an overview of different approaches to macroscale and microscale acoustic particle manipulation by reviewing several typical publications in the field. It should be noted that acoustic particle manipulation can be done in both liquid and gaseous media. The latter is referred to as levitation and is not discussed in this thesis [187-190].

6.3.1 Macroscale devices

Acoustic standing waves are commonly used to increase the local concentration of particles in order to increase the rate of sedimentation and thereby separate particles from their suspending medium. Limaye et al. induced a multi-wavelength standing wave in a cylindrical tube containing suspended yeast cells or Escherichia coli bacteria [191]. Initially the ultrasound was applied continuously while the particles gathered in the pressure nodal planes under the influence of the axial PRF. Further clustering within these planes was most likely caused by the transverse PRF and the SRF. This was followed by a period of pulsed ultrasound, allowing the clusters of cells to sediment before the clarified medium was removed from the upper part of the tube. Several similar devices and methods based on batch sedimentation have been presented [98, 192-198]. Researchers have also used continuous sedimentation methods as acoustic filters in cell culture devices [199-201]. Trampler et al. kept hybridoma cells in a bioreactor and extracted anti-body rich medium through an outlet

which incorporated an ultrasonic retention filter. The cells were forced together in a standing wave field and sedimented back into the bioreactor, thus eliminating a loss of cells during the harvesting procedure. Similar continuous methods have been presented by other researchers [202-204], e.g. Peterson el al. performed continuous separation of red blood cells from plasma by utilizing a semi-standing wave. Whole blood entered a chamber in which the red blood cells were forced to one side where sedimentation took place. The sedimented red blood cells exited through an outlet in the bottom of the chamber while the clarified plasma exited through an outlet close to the top.

Sedimentation methods depend on gravity in order to work and are relatively slow. To avoid these limitations, continuous laminar flow separators with flow splitters can be used. A separator with an h-shaped separation channel, where particles were moved to pressure nodal planes parallel to the direction of flow, was presented by Benes et al. [205, 206]. The rectangular flow channel was expanded in one direction and the flow profile was then divided by a flow splitter. By balancing the outlet flow rates a stream of particle free medium could be extracted at the outlet corresponding to the expanded part of the channel since the particles followed the pressure nodal planes through the wide part of the channel and exited through the opposing outlet. Continuous flow acoustic particle separation devices utilizing flow splitters in combination with laminar flows are very common and have thus been described by a large number of researchers [183, 184, 207-213]. Most commonly, the axial PRF moves the particles into one or more bands in the direction of the flow as the suspension flows through a resonance cavity. Flow splitters located in the end of the cavity divide the flow into a number of outlet channels and by balancing of the flow rates the particle bands can be directed to specific outlets while the particle free medium exits through the complementary outlets. This type of devices are not only capable of separating suspended particles from their medium, they can also separate particles from each other based on size, density, compressibility or a combination of these parameters, as stated by the equation describing the axial PRF (equation 4.10) [183, 184, 211, 213]. Larger particles will for example be more affected by the axial PRF compared to smaller ones and subsequently move faster to a nodal or an anti-nodal plane. Thus, by the use of flow splitters and balanced outlet flow rates it is possible to separate large particles from small ones. Likewise, it is possible to separate particles of the same size if they differ in compressibility and/or density since this affects the ϕ-factor. This mode of operation represents an attractive way of implementing acoustic continuous flow separators but the systems must have a relatively thin fluid layer in order to maintain strictly laminar flow characteristics, a fact that limits the throughput.

In another approach to acoustic particle separation, by Feke et al., a flow chamber filled with a porous medium was utilized [214-216]. Unconsolidated beads formed from glass spheres, aluminium meshes or polymeric foam was used as a porous stationary phase. As the suspended particles passed through the porous medium, secondary forces were believed to have caused the particles to stick to its surface. The particles were trapped for as long as the ultrasound was turned on. When the ultrasound was switched off, the particles were released and washed away. The fact that the porous medium eventually got saturated limited the separation performance.

The same type of device was also used to separate oil droplets from aqueous emulsions [217].

A device capable of separating two particle types based on their speed of response to an acoustic standing wave field was proposed by Mandralis et al. [182, 218]. The speed of response is mainly determined by the magnitude of the axial PRF and the drag force. A combination of frequency and flow direction switching was utilized. Particles that responded quickly to the acoustic field moved faster towards the pressure node in the high flow rate zone in the middle of a flow channel actuated at its fundamental resonance frequency, compared to particles that responded slowly. Thus, the particles that responded quickly generally moved a longer distance along the direction of flow. After a while the actuation frequency was switched over to the second harmonic and two pressure nodes were formed instead of one, thereby moving the quick response particles away from the high flow rate zone of the channel as the direction of flow was reversed. Using this principle, batch or continuous separation could be realized.

Particle traps utilizing acoustic standing waves are often used to increase the local concentration of particles or to catch particles from a continuous flow, e.g. in order to detect them [219-223]. For example, Hawkes et al. captured spores on an antibody coated surface by forming a pressure node close to it [223].

6.3.2 Microscale devices

The microscale domain is ideal for acoustic particle manipulation. The laminar flow characteristics are favourable, making flow splitter based fractionation systems efficient in continuous flow devices. Small structures can be fabricated with high precision using microfabrication techniques, thus offering reduced resonator dimensions and consequently higher resonance frequencies that lead to a stronger axial PRF, capable of moving smaller particles. On the other hand, as the particles become smaller, the axial PRF decreases rapidly. The particle sizes that are likely to be manipulated in microscale devices range from a few tenths of micrometers to tens of micrometers. In this case, the upper size limit is set by practical aspects such as sedimentation and channel blocking. Typical channel dimensions in the standing wave direction in acoustic particle manipulation devices range from a millimetre to a few tens of micrometers, which approximately correspond to operating frequencies between 100 kHz and 10 MHz. Microscale devices generally operate in the MHz regime. A further benefit of the miniaturization of acoustic particle manipulation devices is that they can be integrated with other microdevices, forming complete lab-on-a-chip systems for handling and analysis of microparticles.

A device on the borderline between macroscale and microscale was presented by Hawkes et al. [208]. The device was constructed from metal plates that were processed by wire electro discharge machining followed by fine polishing. The separator was realized both as a two and a three outlet continuous separator with a single band of particles. Hawkes et al. also suggested a miniaturized device capable of washing cells or mixing media depending on the magnitude of the acoustic power applied [224]. Harris et al. combined anisotropically wet etched silicon and isotropically etched glass

to fabricate a true microscale separation device [212, 225, 226]. It comprised a single inlet etched through the silicon wafer, a piezoceramic transducer, a flow-through cavity etched in the glass wafer and two outlets also etched through the silicon wafer. The bottom of the glass cavity was flat enough to serve as an acoustic reflector and thereby a half wavelength standing wave could be formed between this surface and the surface of the silicon wafer. Separation was achieved by balancing the outlet flow rates such that the particle band exited through one outlet and a particle free fraction through the other. Kapishnikov et al. presented a microfluidic acoustic separator with embedded opposing transducers for the generation of the standing wave [186]. The device was realized using soft lithography technology.

Several microscale particle trapping devices have also been presented [16, 108, 227-230]. Lilliehorn et al. realized a trapping device with several individual transducers mounted on a printed circuit board [227, 228, 231]. The channel structures were fabricated using SU-8 and a glass sheet formed the reflector layer. Each transducer generated a pressure node in the centre of the flow channel that could be used to trap and enrich particles and cells from a continuous flow. The trapping array was used to hold particles in positions accessible for different fluids via multiple inlets connected to the main channel. Neild et al. combined acoustic particle manipulation with a microgripper [180, 230]. Particles were first positioned in rows at well defined positions and were subsequently retrieved by the microgripper.

It is also possible to combine acoustic forces with other forces in microscale systems. A device utilizing both dielectrophoretic and acoustic forces was presented by Wiklund et al. in an effort to combine high throughput with precision handling of individual particles [232]. The ultrasound was coupled into a microchannel via a refractive element.

The contributions to microscale acoustic particle manipulation by the author and other members of the nanobiotechnology and lab-on-a-chip group at the Department of Electrical Measurements at the Lund Institute of Technology are discussed in chapter 7 of this thesis.

6.4 Viability of bioparticles

A commonly raised question, with regards to acoustic manipulation of biological particles, concerns the potential decrease in viability that could be caused. The reason for this is probably the fact that ultrasound is often used for cell disruption. On the other hand, it is used a great deal more in medical diagnostic applications, where it is believed to be harmless [233, 234]. There are two main differences between these applications, the frequency and the power of the ultrasound. To disrupt cells frequencies typically range from ~20 kHz up to a couple of hundred kHz even though higher frequencies can be used [235]. In contrast, diagnostic ultrasound frequencies generally range between one and ten MHz [234]. This difference is important since cell disruption is based primarily on cavitation, i.e. formation and implosion of bubbles due to strong mechanical forces exerted on the medium particles. The bubbles are formed when the molecules of the medium are ripped apart, creating void

bubbles. When these bubbles implode they generate shock waves, extreme local temperatures and free radicals. However, the power of the ultrasound needed to cause cavitation above ~100 kHz is substantial and increases very rapidly with increasing frequency [70, 236]. Consequently, ultrasonic waves in the MHz regime can generally not be expected to cause cavitation and should therefore not have a significant negative effect on the viability of biological particles provided that the devices are operated correctly and the power is reasonably low [199]. The power limitations are also important in order to avoid excessive heating. In addition to power, the viability is also affected by exposure time and mechanical properties of the cells determined e.g. by age [237]. In most flow through acoustic particle manipulation devices the exposure time is very short, typically less than a second.

The biological effects of acoustic particle manipulation have been investigated in detail by several researchers. In particular, the viability of red blood cells has been studied since many devices target separation of blood cells [238-242]. No significant damage to the cells was reported at frequencies between 0.5 and 3.5 MHz with the exception of cases where excessive power caused cavitation at frequencies below 1 MHz. The viability of several other cell types has also been investigated at frequencies as low as 200 kHz [196, 231, 243-246]. Again it was found that no significant damage could be detected. Finally, it should be noted that the pressure nodal plane of a standing wave, where most biological particles end up, is a very advantageous place to be when it comes to viability since the pressure amplitude is at its minimum there, i.e. the mechanical stress on the particles is minimal.

7 The Lund acoustic particle manipulation toolbox

The Lund acoustic particle manipulation toolbox offers a number of microfluidic methods that can be used independently or in combination to solve various suspended particle handling tasks. They can also be integrated with other microdevices to form complete lab-on-a-chip systems. This is the first doctoral thesis on acoustic particle manipulation presented by the nanobiotechnology and lab-on-a-chip group at the Department of Electrical Measurements at the Lund Institute of Technology. Hopefully, this work will be continued with the addition of new methods and modifications of the ones presented here.

The basic acoustic particle separation method, with its applications in blood handling, is described in the first part of this chapter [177, 178, 242, 247-252]. When presented, this method was a breakthrough in the sense that it combined acoustic particle manipulation with microfluidics and offered a new and simple way of actuation in combination with visual access to the actual separation process. Soon it was realized that the basic method could be further developed to do a great deal more than just separate particles from parts of their suspending medium or separate two particle types from each other. The resulting additional methods include medium exchange, binary particle switching, frequency switching separation and free flow acoustophoresis (FFA) [252-259].

The author's contributions to the toolbox are the particle separation (7.1), medium exchange (7.2) and free flow acoustophoresis (7.5) methods. In addition, he acted as advisor for the medium exchange through side-washing (7.2.1), binary particle switching (7.3) and particle separation through frequency switching (7.4) methods.

7.1 Particle separation

The Lund method for acoustic separation of suspended particles from their medium is based on a laminar flow microchannel that is ultrasonically actuated from below, using a piezoelectric ceramic (figure 6.2) [177, 178]. The width of the channel is chosen such that a half wavelength standing wave is formed between the side walls. As suspended particles with a positive ϕ-factor flow through the channel, they are moved towards the pressure nodal plane along the centre of the channel by means of the axial PRF. Correspondingly, negative ϕ-factor are moved towards the anti-nodal planes close to the side walls (figure 7.1) [247]. Since the end of the separation channel is split into three outlet channels the positive ϕ-factor particles exit through the centre outlet while the negative ϕ-factor particles exit through the side outlets (figure 7.2). The separation efficiency of positive and negative ϕ-factor particles is defined as the fraction of the intended particle type exiting through the centre and side outlets, respectively.
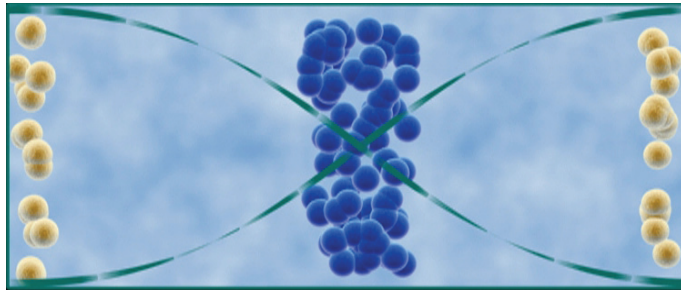
Figure 7.1: *Illustrated cross-section (along the dashed line in figure 7.3) of a separation channel showing negative ϕ-factor particles (yellow) gathered in the pressure anti-nodal planes by the side walls and positive ϕ-factor particles (blue) gathered in the pressure nodal plane in the middle of the channel.*
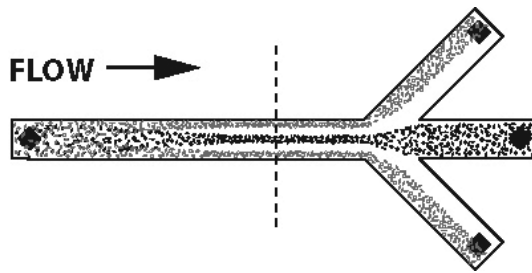

Figure 7.2: *Illustration of the separation of positive ϕ-factor particles (black) and negative ϕ-factor particles (grey) in a chip design with three outlet channels.*

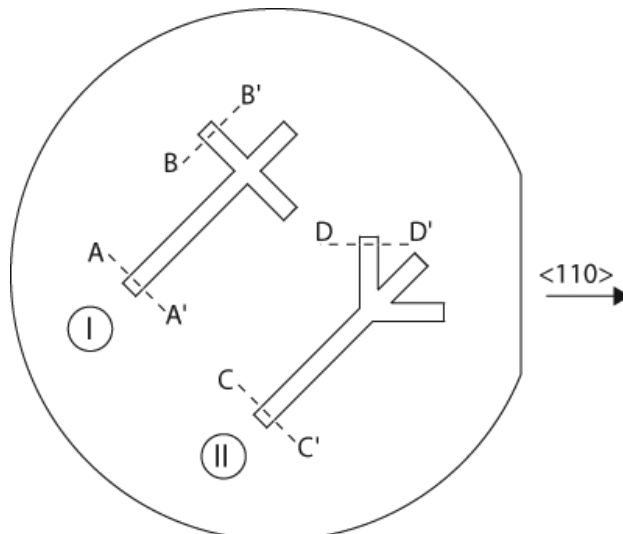

Figure 7.3: *Orientation of 90° (I) and 45° (II) designs on a {100} oriented silicon wafer. The cross-sections A-A', B-B' and C-C' are rectangular while D-D' is trapezoid. The arrow indicates the <110> direction.*

Early chip designs comprised a simple cross structure etched in a {100} oriented silicon wafer such that the separation channel walls were {100} surfaces (figure 7.3 - I) [177]. Conventional anisotropic wet etching thereby provided absolutely vertical and smooth side walls in the separation channel, which was made ~750 μm wide and ~250 μm deep, the prior dimension corresponded to the fundamental resonance frequency, i.e. ~1 MHz in water. However, in operation, the axial PRF was found to be too weak to efficiently move 5 μm polyamide spheres (positive ϕ -factor) to the middle of the channel. To increase the force the frequency was doubled (~2 MHz), i.e. the second harmonic was used. This resulted in a single wavelength standing wave (figure 7.4). At ~2 MHz the polyamide particles gathered in the pressure nodal planes one quarter of the channel width from each side wall and subsequently exited the system through the side outlets when the flow rates in all outlets were identical. Stagnant zones were observed in the early chip designs with outlets diverging 90° from the main channel (figure 7.5). A second design, offering more harmonic fluid dynamics, comprised side outlet channels diverging 45° from the main channel direction (figures 7.3 - II and 7.5). Using this chip design ~90% of the suspended particles were collected in 2/3 of the original medium volume (total flow rate: 0.1 ml/min, actuation frequency: ~2 MHz, actuation voltage: ~15 V_{PP} [pp = peak-to-peak], particle concentration: 2% by volume) [177].
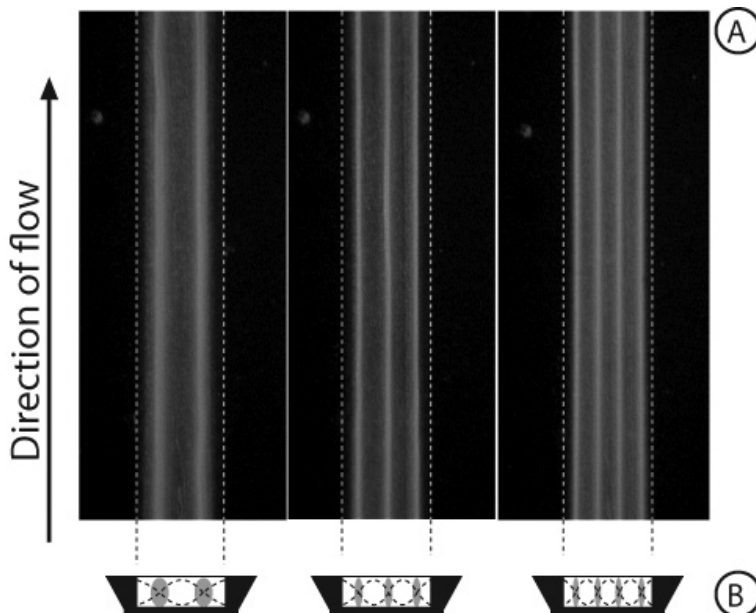

Figure 7.4: Microscope image (A) of 5 μm polyamide particles aligned in parallel bands in a ~750 μm wide channel at different actuation frequencies. Left: second harmonic (~2 MHz), middle: third harmonic (~3 MHz), right: fourth harmonic (~4 MHz). Illustrated channel cross-section (B).
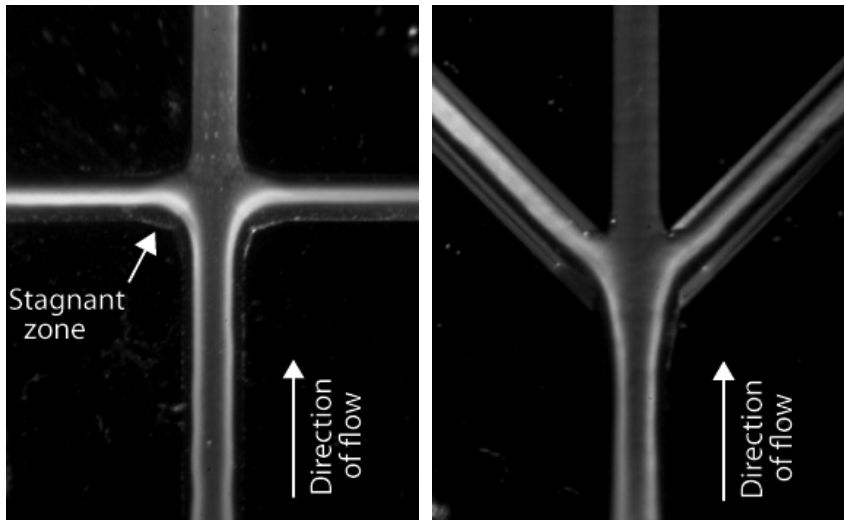
Figure 7.5: *Microscope image of 5 μm polyamide particles exiting a 90° (left) and 45° (right) design chip through the side outlets (second harmonic, ∼2 MHz). Note the stagnant zones (left).*

Since the ∼2 MHz axial PRF was sufficient to move 5 μm polyamide particles, a chip design with the corresponding fundamental resonance frequency was fabricated [247]. In this design, the channel width was ∼350 μm (depth: ∼125 μm), corresponding to half a wavelength in water at ∼2 MHz. Consequently, the polyamide particles now exited through the centre outlet while most of the suspending medium exited through the side outlets, provided that the flow rates in all outlets were identical. When the voltage applied to the piezoelectric ceramic was increased, the acoustic force also increased and the particles were gathered in a narrower band. Virtually all particles could be made to exited through the centre outlet together with 1/3 of the suspending medium (total flow rate: 0.3 ml/min, actuation frequency: ∼2 MHz, actuation voltage: ∼12 V_{pp}, particle concentration: 2% by volume). The maximum applicable voltage was limited by heating of the chip due to attenuation and in rare cases by trapping of particles in the flow channel, the latter most likely an effect of e.g. resonances along the length of the channel, non-uniform amplitude distribution of the source (near field) or influence of the boundaries. If the flow rate was increased the particle band became wider since the particles resided in the standing wave field for a shorter period of time and consequently fewer particles reached the centre of the channel before exiting [247]. A higher concentration of suspended particles also had a negative effect on the separation efficiency since more particles demand more energy in order to be separated and because the centre outlet was overloaded [247].

Fabrication of separation chips using DRIE was also investigated. Since DRIE is crystal lattice independent it offers considerably more freedom in chip design. Unfortunately, this freedom comes at a prize; it proved exceedingly difficult to realize the smooth and parallel wall surfaces that are essential in order to achieve a good resonance in the separation channel. However, a very attractive alternative is to wet etch the separation channel and etch the rest of the system using DRIE, thereby enjoying atomic level precision in the separation channel and the freedom of design in the rest of the system.

The Lund method is a generic continuous flow separation platform offering low mechanical stress and non-contact handling of micrometer sized suspended particles. In theory, the only prerequisite is that the axial PRF is non zero, which is the case if the ϕ-factor is non-zero. Though, by manipulating the density of the medium the contrast factor can be altered to some extent [254, 255]. Heating of temperature sensitive samples due to attenuation in the piezoelectric ceramic actuator and in the bulk material of the resonator can be reduced by cooling the chip, e.g. with a Peltier element.

7.1.1 Lipid particle removal from blood

One of the first applications of the Lund method targeted blood washing in open heart surgery [242, 247-251]. In general, blood shed in the chest cavity during surgery, e.g. coronary bypass, is collected and returned to the patient via a filter, i.e. autotransfused. This blood is commonly contaminated by triglycerides from adipose tissue undergoing surgery. However, when autotransfusion is performed, millions of small fat particles are returned to the patient's circulatory system, resulting in microembolization of the capillary networks of the bodily organs and subsequent local ischemic tissue damage [260-262]. This is most obvious with regards to the brain since the result is noticeable to the patient and people around him or her [263-270]. Elevated levels of cognitive dysfunction have been linked to lipid microembolisation [271-273]. Unfortunately, the techniques currently available, i.e. filtration and centrifugation, are inadequate when it comes to removing fat particles satisfactorily [274, 275]. However, autotransfusion is clearly preferred, in spite of the risk of microembolization, since returning the patient's own blood reduces the strain on the blood banks and results in a shorter convalescence [276-278]. In addition, it eliminates transfusion transmitted disease, immunologic reactions and the risk of blood group incompatibility [279]. When the blood loss is extensive, blood wash devices based on centrifuges are often used [280, 281]. These are, however, burdened with a number of additional drawbacks. They can generally only handle large volumes of blood, expose the blood cells to harmful mechanical stress (high g-levels) and are often non continuous [282].

Fortunately, lipid particles display a negative ϕ-factor while it is positive for red blood cells (erythrocytes). This means that, while passing through a ~350 μm wide separation channel actuated at ~2 MHz, lipid particles will move towards the side walls and exit the system via the side outlets while erythrocytes move to the centre of the channel and exit through the centre outlet (figure 7.6). The fraction of the suspending medium that exits the system together with the lipid particles will be determined by the flow rates set for the different outlets.
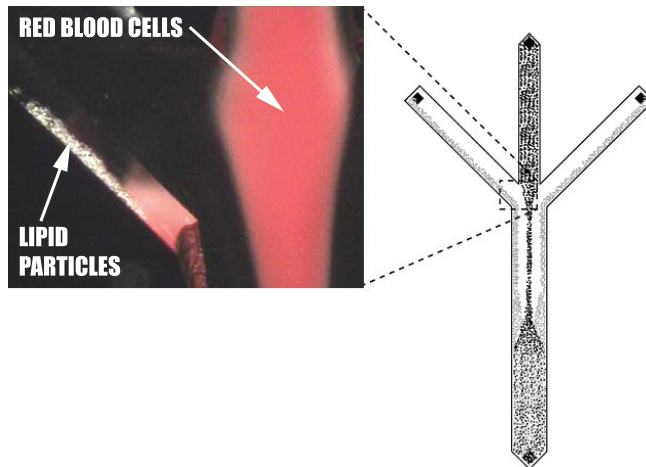
Figure 7.6: *Microscope image showing human lipid particles being separated from red blood cells. A reflection of the red blood cells in the sloping side wall of the left side channel can also be seen.*
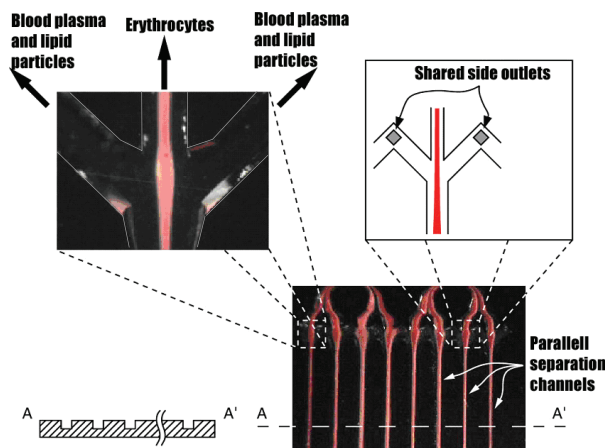
Figure 7.7: *Microscope images and illustrations of a separation chip with eight parallel separation channels. Total flow rate during normal operation: ~1 ml/min.*

In a study on lipid microemboli separation, a sonicated emulsion of tritium labeled trioleine in saline solution (9 mg/ml) was used to evaluate the lipid particle separation efficiency (lipid particle size: $\geq$ 0.3 μm). The measurements were performed by detecting the radioactivity of the waste (side outlets) and enrichment (centre outlet) fractions. It was shown that about 85% of the lipid particles were removed more or less independently of the concentration of erythrocytes, while the erythrocyte recovery decreased at elevated red blood cell concentration levels (total flow rate: 0.3 ml/min, actuation frequency: ~2 MHz, actuation voltage: ~10 V_{pp}, erythrocyte concentration: 2.5-10.0% by volume, lipid concentration: 1% by volume) [247]. The concept of increasing the throughput by using several parallel channels was later demonstrated (figure 7.7) [242, 283]. The mean separation efficiency, for both erythrocytes and lipid particles, was found to be roughly 80 % in the investigated concentration range (particle concentrations: 5-30 % erythrocytes and 0.5-2.0 % lipid particles by volume,

eight parallel channels, centre outlet flow rate: 0.5 ml/min, total side outlet flow rate: 0.5 ml/min, actuation frequency: ~2 MHz, actuation voltage: 18-28 V_{pp}) [242]. A parameter to account for regarding the erythrocyte separation efficiency was overloading of the centre outlets at elevated red blood cell concentration levels. The first step in improving the characteristics comprises balancing of the waste and enrichment flow rates. The implementation of serially linked separation steps can further improve the situation [283]. Figure 7.8 shows the basic design of a chip for serial processing in three steps.
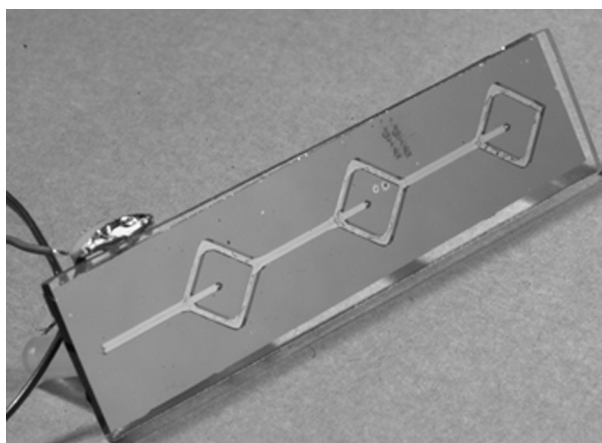

Figure 7.8: *Three step serial separation chip, e.g. for processing of highly concentrated samples with minimal loss of particles.*

In addition to separating lipid particles from erythrocytes, the separation method has a clear potential in handling other blood component therapy and analysis tasks, e.g. to produce clinically clean plasma [249, 284, 285]. It has been shown that the concentration of red blood cells can be reduced from ~40% to <1% in a four step separation process with eight parallel separation channels (total flow rate: 0.6 ml/min) [249].

7.1.2 Increasing throughput

A generally raised concern regarding particle separation performed in the microchip format is the limited throughput. This is something that is inherently linked to the microscale environment in which the processes occur. On the other hand, microscale is generally also a prerequisite to be able to capitalize on the favourable scaling laws that offer beneficial physical properties, e.g. laminar flow. In regards to acoustic standing wave based particle separation, the scaling laws favour downscaling since it becomes possible to employ a strong axial PRF in a controlled fashion. As the width of the separation channel is reduced the fundamental resonance frequency is raised, which increases the magnitude of the PRF. Thus, the microscale benefits are evident and fundamental to the separation performance. However, when designing systems for analytical purposes, low throughput is generally not a problem. On the other hand, a major part of the anticipated applications are seen in fields where high throughput is desired, e.g. as in the cases of blood washing during surgery and in post surgery blood

salvage. Obvious applications can also be seen in blood banking, where blood component fractionation is a major task with throughput requirements of litres per hour or higher [140]. An approach to meet these demands, which yet comply with the basic ideas of miniaturisation, is to implement a design that holds identical parallel channels connected in e.g. a bifurcation structure. Figure 7.9 shows the layout of an eight channel separation chip.

A clear benefit of the Lund method is the fact that the volumetric throughput can be improved by increasing the number of parallel channels using the same piezoelectric actuator. Whereas the original single channel separator typically offers throughputs in the order of 0.1 ml/min the eight-channel separator offers flow rates in the order of 1 ml/min [242, 249, 283].
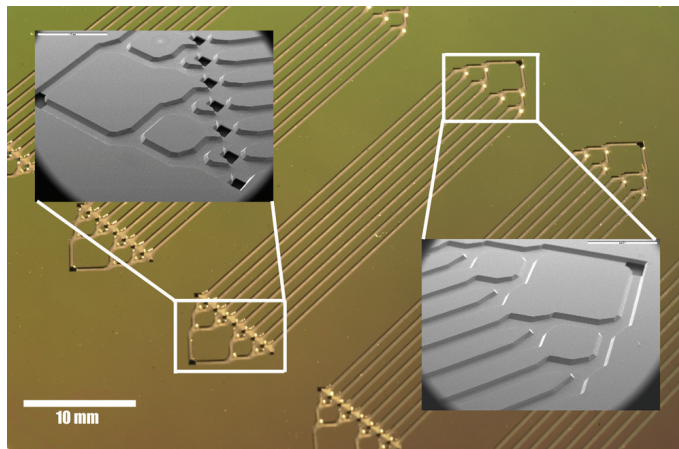
Figure 7.9: Picture of several separation structures with eight parallel channels. Scanning electron micrographs showing details of the channel outlets (left) and inlet (right). The row of black diamond shaped structures is the waste outlets (left insert) that are connected to a common waste outlet on the back side of the chip. The single outlet at the far left is the enrichment outlet.

7.2 Medium exchange

Laminar flow microchannels offer the possibility to laminate different liquid media in a streaming system. This, in combination with acoustic particle manipulation, can be utilized to move particles from one suspending medium to another by means of the axial PRF [224, 252, 253]. A particle suspension enters a rectangular cross-section microchannel, similar to the one used to separate lipid particles from erythrocytes, through two side inlets (figures 7.10 – 7.12) [253]. As a second, particle free, medium enters through a single centre inlet it is laminated between the side inlet streams. If the particles have a positive ϕ-factor they will move from their original medium into the medium originating from the centre inlet when a half wavelength standing wave is applied and subsequently exit through the centre outlet. The original medium exits via the two side outlets. Analogously, in cases where the particles have a negative ϕ-factor, they are instead supplied via the centre inlet and are subsequently switched over to a second medium entering through the side inlets.
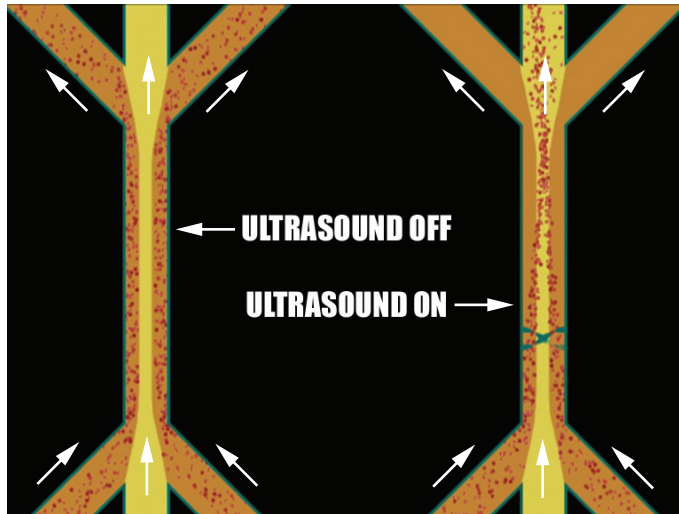
Figure 7.10: *Schematic illustration of the medium exchange principle. When the ultrasound is turned off the particles enter through the side inlets and exit through the side outlets together with the contaminated medium. If the ultrasound is turned on the particles are switched over to the clean medium and exit through the centre outlet.*
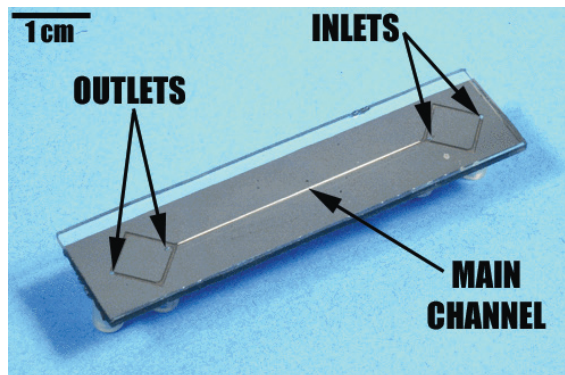
Figure 7.11: *Front view of a medium exchange chip.*
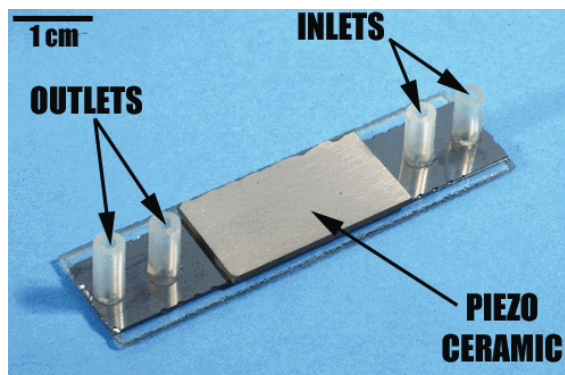
Figure 7.12: *Back view of a medium exchange chip with a piezoelectric ceramic actuator attached.*

When two media are laminated some mixing will always occur due to diffusion. In the case of continuous particle washing, diffusional mixing may induce undesired cross-contamination between the laminated streams. However, this problem can be eliminated by balancing the exit flow rates such that the part of the particle free medium closest to the contaminated medium is directed to the side outlets. When accounting for mixing effects, Rayleigh mixing should be considered since it can be important at very low flow rates. However, during separation of micrometer sized particles and at the flow rates commonly used, Rayleigh mixing is usually not a problem.

The medium exchange method was first demonstrated by moving 5 μm polyamide particles from a medium spiked with Evans blue to a clean medium. It was shown that ~95% of the contaminant could be removed (flow rate: 0.1 ml/min through the side inlets and centre outlet, 0.17 ml/min through the centre inlet and side outlets; actuation frequency: ~2 MHz; actuation voltage: ~10 V_{pp}; particle concentration: 1.5% by volume; contaminant concentration: 360 μg/ml) [253]. Similar medium exchange efficiencies were obtained when erythrocytes were translated from whole blood, spiked with Evans blue, to clean blood plasma. It should be noted that the medium exchange efficiency was found to decrease with increasing particle concentration. The sources of this contamination are assumed to be the hydrodynamic drag from each particle transiting the media boundary and contaminants weakly adsorbed on the surface of the particles.

The described medium exchange method can be used to extend the applicability of the blood wash method to include post surgery blood recycling, i.e. when the patient is bleeding after surgery and the shed blood is collected via a drain tube placed in the chest cavity. In this case the blood plasma can be heavily contaminated by drugs, immunologically active substances and coagulation factors that should not be returned to the patient [276, 286-289]. To avoid this, the erythrocytes can be moved from the contaminated plasma to clean plasma or saline solution for safe reinfusion.

7.2.1 Medium exchange through side–washing

A complementary medium exchange method, termed side-washing, was also developed [258, 290]. The advantage of this method, compared to the standard method, is that it is less sensitive to differences in the properties of the two media. When using the original method, particles were sometimes observed to have difficulties in passing the boundary layer between the two media. In both methods the particles are focused in the middle of the flow channel by the axial PRF but in side-washing they are not moved from one medium to another. Instead, the new medium is stepwise added from consecutive inlets along one side wall of the channel while the old medium is removed from outlets along the opposing side wall (figure 7.13). Due to the laminar properties of the system a step-wise side shifting of the old medium occurs. The purpose of the acoustic force is to prevent the particles from leaving the system together with the contaminated medium. Chip designs with up to eight side shifts were investigated. About 97% of the contaminant could be removed while recovering ~80% of the particles entering the system (total flow rate: 0.1 ml/min, side

shift flow rate: 20-30 µl/min per junction). The lost particles exited through the outlets along the side wall. The medium exchange efficiency can be increased even further if the particle recovery efficiency is less important, e.g. >99% of the contaminant can be removed if it is acceptable to loose 70-85% of the particles.
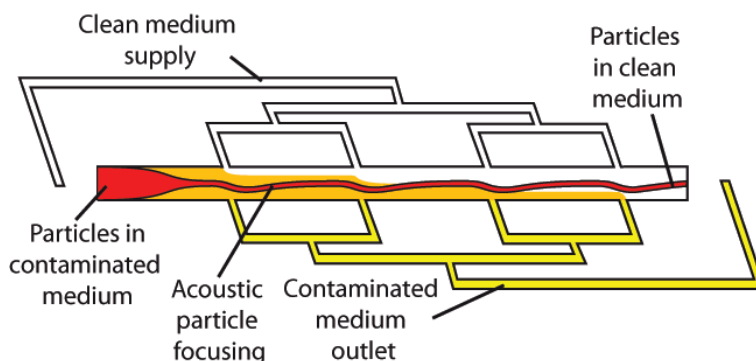
Figure 7.13: *Schematic illustration of the side-washing medium exchange principle. A clean medium is added through inlets on one side while the contaminated medium is removed through outlets along the other. Meanwhile, the particles are held in place by the axial PRF.*

7.3 Binary particle switching

The combination of the axial PRF and laminar flow microchannels can also be used to realize valve-less particle switches for controlled distribution of particles in microfluidic networks [256]. Today, this is most commonly done through dielectrophoretic methods [135]. However, it is possible to utilize the described medium exchange chips in binary switching networks. Unfortunately, the design does not allow this to be done in one plane since the centre outlet must be connected to one of the succeeding inlets via an out-of-plane U-link. The design can, however, easily be modified to allow in-plane switching networks. This is done by using only two inlets and two outlet channels (figure 7.14). Several of these switches can then be interconnected in order to solve particle distribution tasks. A four outlet chip design is described in figure 7.15 and the four binary settings of the chip in operation are shown. In this configuration transducer C1 was operated at ~1.9 MHz (~380 µm wide channel) and transducer C2 at ~1.6 MHz (~420 µm wide channel).
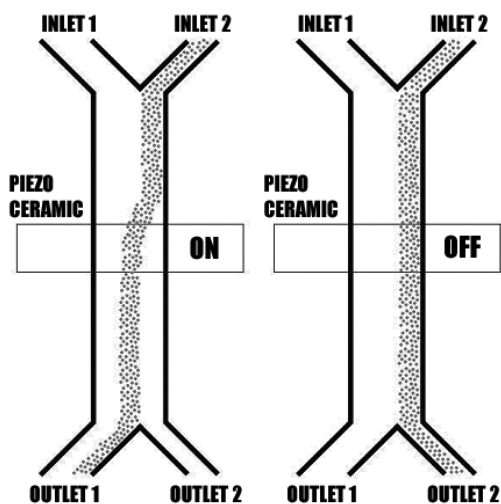
Figure 7.14: *Binary switching principle. Suspended particles enter through inlet 2 and a particle free medium enters through inlet 1. By switching the transducer on and off it can be decided whether the particles are to exit through outlet 1 or 2.*
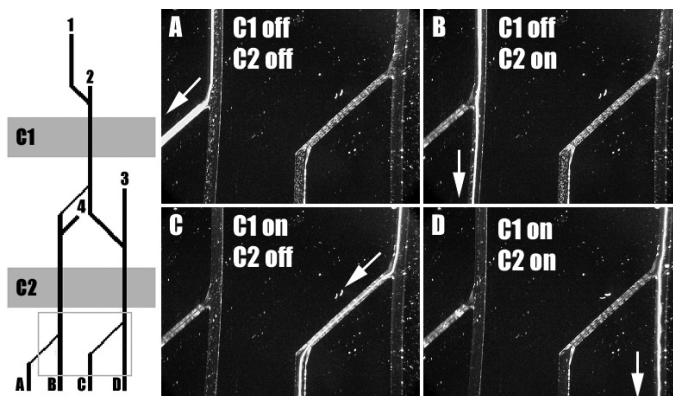
Figure 7.15: *Left: One possible design of a four outlet wet etched binary switching tree. Suspended particles enter through inlet 1 while a particle free medium enters through inlets 2, 3 and 4. By turning the piezoelectric transducers, C1 and C2, on and off, in a binary code sequence, the particles can be directed to one of the four outlets, A-D. Right: Microscope images of the four possible settings (5 μm polyamide particles). The field of vision corresponds to the grey square in the figure to the left.*

For binary particle switching trees to reach their full potential some aspects must be considered. First of all, it is essential to be able to operate one switch independently of the others. This can be done, as above, by driving the piezoelectric ceramic actuators at non interfering frequencies or by isolating them acoustically from each other. The latter can be done either by mounting several switching chips in a rig with interconnecting tubing or by attenuating the acoustic coupling between the actuators in the switching network. Secondly, the parabolic flow profile causes problems when the sample particles flow through the switching trees since those travelling close to the

top and bottom of the channel will travel slower than the ones in the centre. This can be addressed by applying a second standing wave, between the top and bottom of the channel, in order to keep the particles closely together in that dimension. However, lagging particles will not be a problem if the sample volumes injected are small compared to the total volumetric flow rate and the number of consecutive switches is low. The binary switching method inherently opens up the possibility of realizing automated on-chip rare event sorting, which has been proven to work in principle [291]. By defining an optical inspection point, single particles with a characteristic optical property, e.g. a fluorescent label, can be selected from a dilute stream of particles by activating the switch. This mode of operation has clear implications in cell sorting applications. When adapting the acoustic switching method to the selection and counting of cells, identified by fluorescently labelled cell specific antibodies, an on-chip fluorescent activated cell sorting (FACS) system can be realized. It may even be possible to develop on-chip multiplex FACS-systems that provide higher degrees of differentiation than current FACS-systems [292]. Figure 7.16 (left) shows the principal operation of an optically triggered particle selection based on the acoustic switching principle. Figure 7.16 (right) indicates the power of the chip integrated format in the sense that high levels of particle differentiation can be realized. A frequent argument with respect to chip based microfluidic FACS-systems is that the throughput is commonly orders of magnitude lower than what can be accomplished with conventional FACS-systems. The acoustic approach, however, displays relatively rapid switching of particles, typical transit times to the centre of the channel is in the order of 10-20 ms. The fact that the concept is prone to massive parallelization also opens up the possibility of increasing throughput at least one order of magnitude.
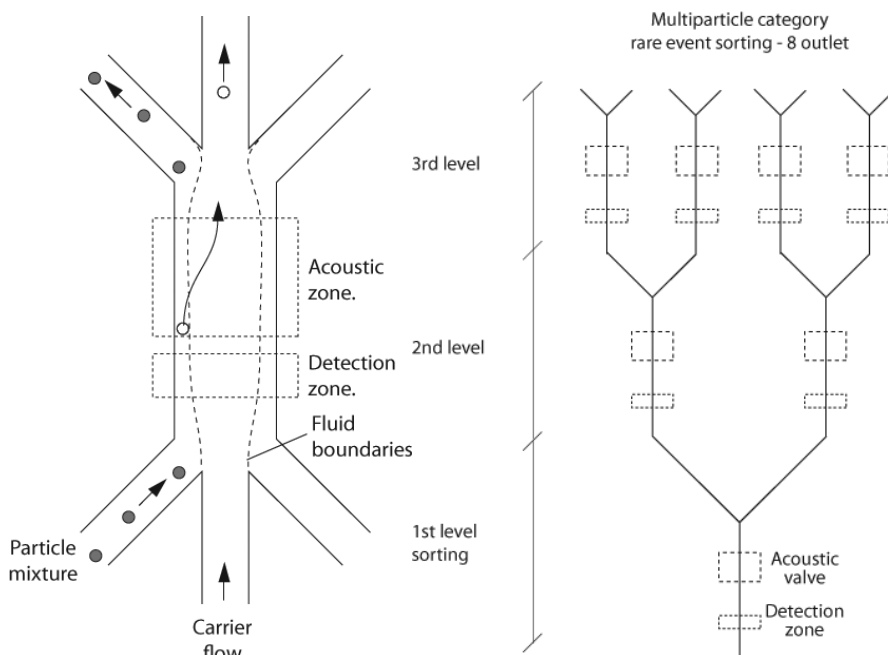

Figure 7.16: *Principle design of rare event particle sorting based on acoustic switching (left). Schematic layout of an eight outlet acoustic switching system in three levels for rare event sorting (right).*

7.4 Particle separation through frequency switching

By alternating between different channel resonance frequencies, matching the n·λ/2 criteria, particles can be separated [257]. This is believed to be possible since the magnitude of the axial PRF differs between dissimilar particle types and particle positions in the standing wave. In the simplest case large and dense particles (positive ϕ-factor) are mixed with small and light (positive ϕ-factor) and enter the separation chip in figure 7.11 through the side inlets while a particle free medium enters through the centre inlet. At time T1 (figure 7.17) a single wavelength standing wave (second harmonic, ~4 MHz) is applied and thus the particles move towards the pressure nodal planes one quarter of the channel width from each side wall. The large and dense particles move faster because they are affected by a stronger axial PRF. When switching over to the fundamental resonance frequency (half wavelength, ~2 MHz) the particles will start to migrate towards the pressure nodal plane in the centre of the channel instead (T2). If the frequency is switched back at the right moment the larger particles will be located close to the centre of the channel, where the axial PRF is at its minimum (T3). On the other hand, the smaller particles will have moved a shorter distance towards the centre and are still close to the pressure nodes of the second harmonic. After switching again (T4) the larger particles will be close to the centre while the smaller ones will be at approximately the same position as at T2. If the switching continues the larger particles will end up in the middle of the channel and the smaller ones one quarter of the channel width from each side wall (figure 7.18 and 7.19). Using this method, it was demonstrated that, when separating 3 µm polystyrene (1.05 g/cm³) and 8 µm polymethylmethacrylate (1.22 g/cm³) particles, at least 80% of the particles could be collected in the intended outlets. The fundamental resonance and the second harmonic were typically active for 800 and 200 µs respectively and the total flow rate was 90 µl/min.
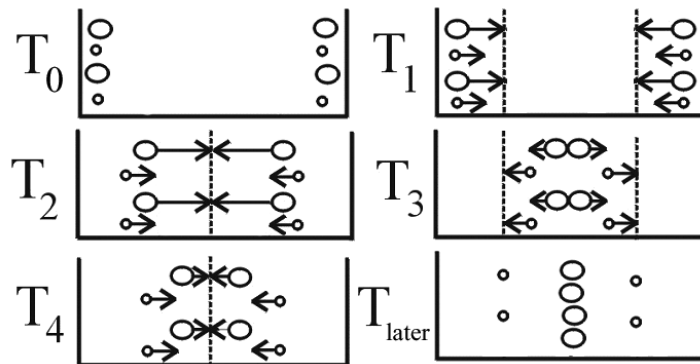
Figure 7.17: *Frequency switching separation principle. T_0 is the situation before the frequency switching starts. At T_1 and T_3 the frequency corresponds to the second harmonic of the channel (two pressure nodes). At T_2 and T_4 it corresponds to the fundamental resonance (one pressure node). When exiting the system the particles will have reached relatively stable positions (T_{later}).*
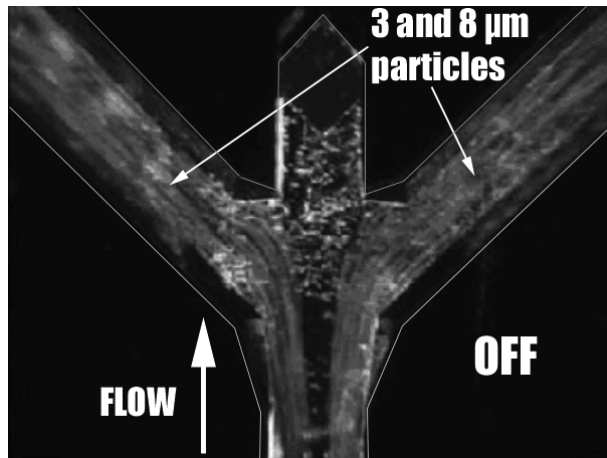
Figure 7.18: *Microscope image of the situation when the ultrasound is turned off. Both the 3 μm and the 8 μm particles exit through the side outlets.*
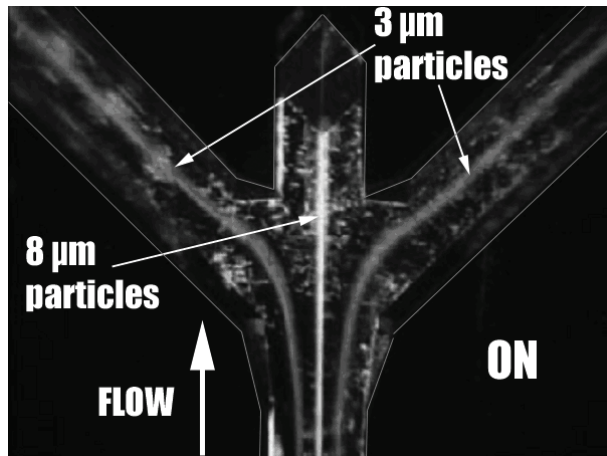
Figure 7.19: *Microscope image of the situation when the ultrasound is turned on. The 8 μm particles are focused in the fundamental resonance pressure node and exit through the centre outlet while the 3 μm particles are gathered in the second harmonic pressure nodes and exit through the side outlets.*

7.5 Free Flow Acoustophoresis (FFA)

Particle suspensions often contain several types of suspended particles. In order to separate these simultaneously or separate one from the others a method termed free flow acoustophoresis (FFA) was developed [254, 255, 259, 293]. The basic concept is to create a gradient of particles, based on their acoustic properties, which can be fractionated in several parallel outlets to achieve fractions dominated by one particle type. The process starts when suspended particles enter a separation channel through two side inlets (figure 7.20). Simultaneously, a particle free medium enters through a centre inlet and the three streams are laminated. As the particles reach the part of the channel that is acoustically actuated at its fundamental frequency they start to move

towards the centre of the channel. Particles that differ in size, density, compressibility or a combination of these factors move at different rates and thereby a particle gradient is formed, provided that the magnitude of the acoustic force is correctly balanced in relation to the flow rate (figure 7.21). The gradient can then be fractionated using a number of outlets and the laminar properties of the flow (figure 7.22). One wet etched chip design with six outlet fractions is pictured in figure 7.23. If it is desirable to extract one particle type from several others a three outlet version of the chip can be utilized. The target particles would in that case exit through the second outlet while particles more or less affected by the acoustic force would exit through the first and third outlets, respectively. Many interesting design alternatives can be realized by combining a wet etched separation channel with a DRIE fabricated fluidic network. In addition to the basic FFA concept, it has been shown that the relative magnitude of the axial PRF acting on different particle types can be changed by adding a density changing substance to the medium entering through the centre inlet, thus enabling the separation of particles that under normal conditions cannot be separated from each other using acoustic methods.
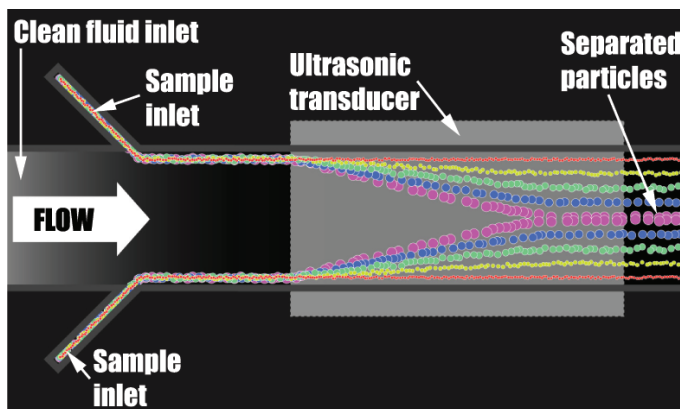
Figure 7.20: *Illustration of a particle suspension passing over the transducer where the particles are moved towards the centre of the separation channel at rates determined by their size, density and compressibility. Because of the laminar flow almost no mixing takes place.*
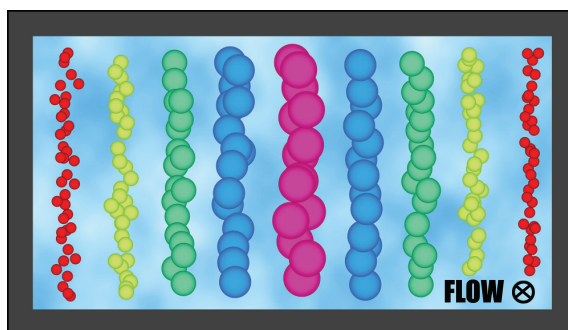
Figure 7.21: *Illustration of the cross-section of the separation channel directly after the transducer. The particles that are most affected by the axial PRF have reached the stable position in the centre of the channel while the others are located somewhere between the walls and the centre.*

When three sizes of polystyrene spheres (3, 7 and 10 µm) were separated into three outlet fractions almost all 10 µm particles, 96%, exited through the centre outlet while 67% of the 7 µm and 81% of the 3 µm particles exited through the second and third outlets, respectively [254]. During the experiment all outlet flow rates were set to 0.13 ml/min and the sample suspension flow rate was 0.04 ml/min. Four particle sizes were also separated with similar results. Furthermore, medium density manipulation was successfully used to separate 3 µm polystyrene and polymethylmethacrylate spheres.

Blood is a complex substance that contains three types of blood cells suspended in blood plasma; red blood cells (erythrocytes, diameter: 7 µm, concentration: 4.2-6.2·10^{12}/L), white blood cells (leukocytes, diameter: 5-20 µm depending on type, concentration: 4.5-11.0·10^9/L) and platelets (thrombocytes, diameter: 2-4 µm, concentration: 1.5-4.5·10^{11}/L) [140, 279]. White blood cells are subdivided into three groups; granulocytes, monocytes and lymphocytes. By combining FFA with medium density manipulation it was shown that it was possible to separate red blood cells and platelets with very good separation results, 92% and 99% of the red blood cells and the platelets, respectively, ended up in separate fractions (total outlet flow rate: 0.20 ml/min, sample suspension flow rate: 0.02 ml/min) [254]. It was also shown that it is likely that continuous and simultaneous separation of red blood cells, white blood cells and platelets is possible, even though a closer study has to be made in order to find the optimum chip design and separation parameters [254].
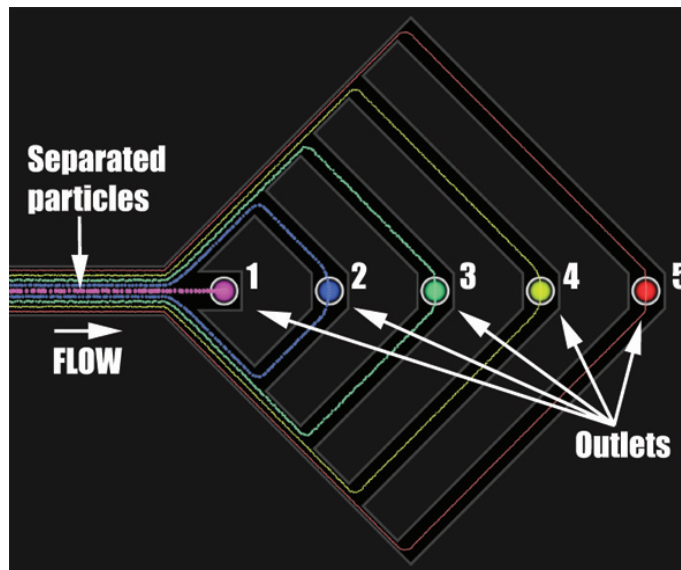
Figure 7.22: *Illustration of the fractionation of the separated particles at the end of the separation channel, in this case through five consecutive outlets. Since the separation is symmetrical along the centre of the channel, eight of the fractionation outlet channels are pare-wise connected.*
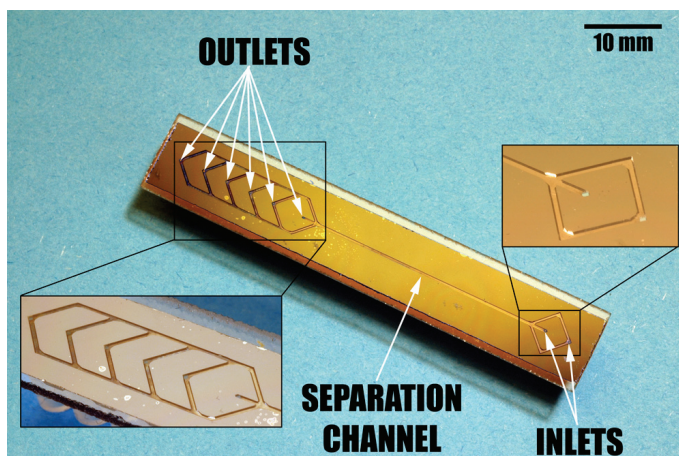
Figure 7.23: *Photograph showing the basic device design, which consists of a silicon chip with wet-etched channel structures. The channels have been sealed with an anodically bonded glass sheet and the inlets and outlets have silicon tubing connections on the back side of the chip.*

To avoid confusion, it should be noted that the term "acoustophoresis" also is used in another context, i.e. when referring to the measurement of the electrical fields induced by ultrasonic waves in electrolytes [294]. The first person to use the term in the context of acoustic particle manipulation was probably Heyman [295]. Since then the term has been used inconsistently in a number of patents and conference abstracts but no journal articles seem to be available. Its use in the definition of FFA should not be regarded as related to any of the previously published definitions of the term.

7.6 Related tools

In addition to the methods described in this thesis there is one more acoustic particle manipulation method, developed by Evander et al. of the nanobiotechnology and lab-on-a-chip group in Lund, which has a potentially important role to play in the particle manipulation toolbox, i.e. an acoustic particle trap [228, 231]. The basic principle is simple, suspended particles move into a cavity in which the bottom surface is a piezoceramic actuator. As the particles enter they are first focused by the axial PRF and then trapped by the transverse PRF in a half wavelength standing wave normal to the surface of the actuator [99]. Once the particles have been trapped the active inlets and/or outlets can be switched e.g. to flush the particles with different reagents or to let them out through a specific outlet. Unfortunately, the current design of this device does not allow on-chip integration but it should be possible to realize such a design. A microfluidic mixing device based on Rayleigh mixing and non-acoustic devices, e.g. microfluidic reactors and dispensers, have also been developed within the group and can be used in combination with the toolbox methods [114, 296, 297].

8. Conclusions

The work presented in this thesis shows that it is possible to use standard microfabrication methods to realize microfluidic devices for acoustic particle manipulation. These are capable of separating different types of suspended micrometer sized particles, biological or non-biological, from each other and/or from their suspending medium. Since standard microfabrication processes and external detachable ultrasonic transducers are used, the microdevices can easily be integrated with others, e.g. to form complete lab-on-a-chip systems. In addition, the throughput can be increased beyond the analytical scale through parallelization in applications where that is required. The concept of medium manipulation was also demonstrated in order to make acoustic particle manipulation possible in situations where it would normally be difficult or impossible.

It was found that the Lund method device design performed well and that it was advantageous in several aspects. First of all, the simple design and the use of microfabrication processes offered the possibility to batch-fabricate several identical devices. This means that production times can be kept short and costs kept relatively low. Depending on the specific design it is doable for one person to manually produce up to about a hundred units in one day and thousands could be produced during the same period of time in an automated industrial scale production line. Secondly, full optical access to the fluidic network offers e.g. supervision of the process and the possibility of event controlled selection. Thirdly, the Lund method offers simultaneous actuation of several parallel channels, either connected for high throughput operation or to enable parallel processing of multiple samples. Fourthly, since the transducer is detachable it can be reused indefinitely. This is an important feature in order to keep costs down in applications where the parts that come in contact with the sample get contaminated and need to be disposable. Fifthly, the behaviour of the devices proved stable. Several different device designs and several devices of each design were used in the evaluation process and very few of these showed an atypical behaviour. Lastly, the fact that the same basic principles already have been used to solve a number of particle handling tasks implies that it is a versatile approach with a great potential to be further developed. Thus, more methods are likely to be added to the Lund acoustic particle manipulation toolbox as the work continues.

The developed devices were used in several blood cell handling applications. First of all, contaminating lipid particles were removed from red blood cells. The use of such a procedure during cardiac surgery could save thousands of people annually from brain damage caused by lipid microembolization and reduce strain on the blood banks. Secondly, it was shown that it is possible to extend the use of the blood recycling method to include post surgery applications by using the medium exchange method to wash away contaminants from the blood cells. These findings open a route to blood recycling in situations where blood is lost today. Thirdly, blood cell free plasma, which can be used in therapeutic applications, was produced in a serial purification process. Fourthly, red blood cells and platelets were separated from each other using a combination of the FFA method and medium manipulation. Lastly, it was shown that

it is likely that parallel and continuous separation of platelets, red blood cells and white blood cells can be performed. Provided that the selectivity and throughput of the FFA process can be increased it could potentially reform the way blood components are separated.

9. Outlook

This is only the beginning! It is not unlikely that acoustic particle manipulation methods will be used routinely in laboratories and/or in the industry within decades. The impressing window of operation with regards to separation parameters, the continuous mode of operation and the adaptability to various applications is almost too good to be true. The device development, in microscale and in macroscale, is by no means finished but it has come far enough to make it likely that the remaining difficulties will be worked out. There are already a few devices commercially available and others will probably reach the market within the next few years.

When it comes to the work presented in this thesis some challenges remain. Most importantly, a dedicated effort should be made to explain the nature of the resonance that is exploited in the Lund method. Even though the devices obviously work very well, a better understanding of the underlying phenomena would give valuable input in the process of optimizing the method. In this context it would e.g. be appropriate to look at the design aspects in the direction normal to the transducer surface, the propagation of the sound waves within the devices and the potential importance of the crystal structure of the bulk material. Further optimization might even make it possible to use cheaper fabrication materials in e.g. disposable applications.

The microfabrication of the devices can be taken one step further by combining wet etched resonance channels with dry etched fluidic networks. This would make new design alternatives available and offer more area conservative design possibilities, which would be beneficial in e.g. lab-on-a-chip systems and high throughput devices.

To be able to quickly integrate the methods of the toolbox, e.g. when developing lab-on-a-chip systems or in temporary set-ups, it would be desirable to have a microfluidic motherboard available. Using a standard interface the individual devices could be plugged in and out to form systems for various complex particle handling tasks. The motherboard would hold the ultrasonic transducers and the fluidic connections and act as a stable rig for the transducer-chip units.

Three major efforts that are being made to extend the applicability of the Lund acoustic particle manipulation toolbox can be disclosed at the moment. Firstly, dispersion caused by the parabolic flow profile in the FFA chips is being reduced through 2D-focusing of the sample particles before they enter the separation channel. This allows for particles that follow close-lying trajectories to be separated and may e.g. offer the possibility to separate different types of leucocytes. Secondly, the acoustic FACS method is moving from the proof of principle stage into the optimization stage. The continued work will focus on applications where acoustic FACS systems can be anticipated to offer advantages compared to conventional FACS systems. Integration with acoustic particle traps in an effort to increase the concentration of rare events will also be investigated. Thirdly, a new separation principle based on the medium exchange method is being developed. The concept, termed Xtract, is in principle capable of extracting specific nucleic acids, proteins or other biomolecules from complex particles suspensions (figure 9.1). The separation

relies on commercially available beads with ligands of a specific affinity attached to the surfaces. The ligands are typically antibodies, proteins, antigens, DNA/RNA probes or any other suitable molecules that bind the target molecules to the beads. After the attachment step the beads are moved from their suspending medium, which may contain other molecules, to a second medium. In the next step the molecules are detached from the beads. Finally, the beads are moved from the second medium to a third medium. The result is in one fraction with no target molecules, one fraction containing only the target molecules and one fraction containing the beads, ready to be used again. Even though Xtract is in its infancy, initial experiments have shown promising results. Nevertheless, provided that the method works as anticipated, it offers a way to realize indirect acoustic separation of molecular sized particles.
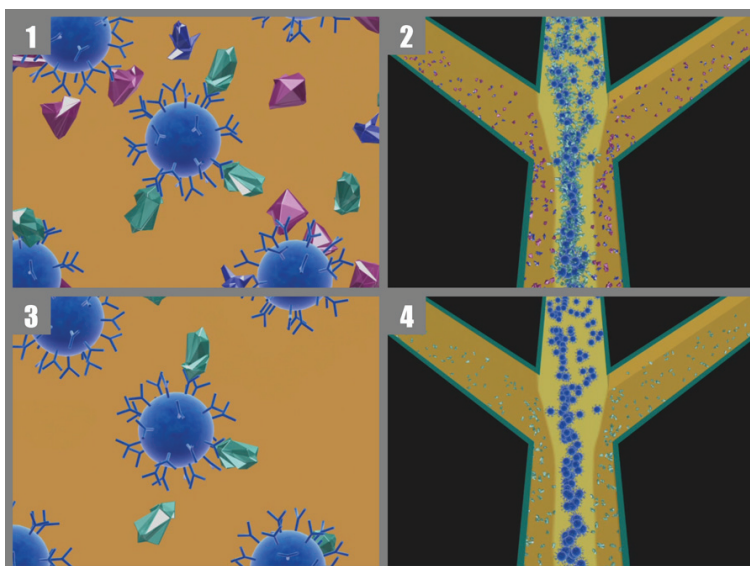
Figure 9.1: *Illustration of the four major steps of the Xtract method. 1: Beads with ligands of a specific affinity binds the target molecules. 2: The beads are separated from other molecular particles through medium exchange. The acoustic force experienced by the molecular particles is negligible compared to that experienced by the beads. 3: The target molecules are detached from the beads. 4: The beads are separated from the target molecules through medium exchange. After this step the target molecules have been isolated and the beads are ready to be used again.*

Last but definitely not least, collaboration projects with researchers in medicine, biomedicine, chemistry, biology and other disciplines should be continued and intensified in order to find more particle handling applications and develop acoustic particle manipulation methods for dealing with them. In this work it would be practical to have standardized criteria for comparison of different particle manipulation methods since it would assist in the identification of the areas of application where acoustic methods offer the greatest additional benefits compared to conventional methods. Though, the main challenge is to make people aware of the fact that powerful acoustic particle manipulation methods exist. I have not yet met a scientist working with cells or other particles that has not reacted with both surprise and genuine excitement on hearing about the methods presented in this thesis.

Acknowledgements

Finally, the goal is within reach! Some months ago, when I started to write the first sentence of this thesis, it felt like the Nordwand of Eiger was in front of me. But mountains are there to be climbed and now I am quite close to the summit. However, before it is time to place the flag it is more than appropriate to take the opportunity to thank a number of people.

First and foremost I would like to thank my supervisor, Professor Thomas Laurell, for years of inspiring and fruit bearing cooperation. I will never cease being astonished by the width of your knowledge, your inexhaustible stream of new ideas and your irritating tendency to be right when I think you are wrong. I am also grateful that you introduced me to hunting, which reminds me of the now legendary words once written about you; "Lusten att döda är inte vad man förknippar med denne vänlige man." (The desire to kill is not what you would associate with this kind man.). Luckily the reporter that wrote it was referring to animals and not PhD-students.

Furthermore I would like to thank all former and present colleagues and students at the Department of Electrical Measurements that I have come in contact with. I am grateful to all of you but some of you deserve a special recognition: Andreas Nilsson, for close collaboration from the first day of this project; Mikael Nilsson, for corridor wrestling matches, balanced opinions and everything but relaxing climbing and skiing holidays in the Alps; Simon Ekström, for profound ideas on how to make this world a better place; Sus Levin, for her happy personality, helpful nature and intelligence reports; Per Augustsson, for injecting new life into the project and for surreal adventures in Tokyo; Josefin Starkhammar, for trying to force me to use skin care lotion; Kerstin Järås, for bringing the art of advanced lunch dishes into the lunch room; Monica Almqvist, for being my assistant supervisor; Johan Nilsson, for late night "whisky club" meetings during conference trips; Anton Ressine, for extensive reviews on everything from philosophers to climbing vacations; Eva Everitt, for kindly helping me with things that I probably should have done myself and for making the corridors smell good; Tomas Jansson, for having a stranger hobby than I have and for letting me practice climbing on his sky reaching piles of papers and books; Lennart Nilsson, for his skilled craftsmanship; Hans Persson, for trying to make me understand things that probably cannot be understood; Magnus Cinthio, for a number of very good naps while getting my blood-vessels checked; Lars Wallman, for telling me what not to do in the clean room after I have already done it and for insight in the world of secret societies; Melker Sundin, Carl Siversson, Carl Grenvall, Mickael Carlsson and Torbjörn Hedberg for their contributions to the acoustic particle manipulation toolbox; David Adolph, for his intriguing opinions on everything between heaven and earth; Everyone else that that I may have forgotten, for having made my years at the department worthwhile.

In addition to my colleagues at the technical faculty I would like to thank Henrik Jönsson, Cecilia Holm, Lena Åberg, Ann-Margret Swärd-Nilsson and Ulf Johnsson at the medical faculty for their important contributions to the medical applications in this project.

I would also like to thank the team at ErySave AB for being good colleagues and for making it a joy to come to work. A special recognition goes to my boss, Thomas Hertz, for never ever making me feel a conflict of interests between my role at the company and my role at the department.

Last but definitely not least, I would like to thank my family and friends for supporting me throughout these years. It has meant virtually everything for me and this would certainly have been much more difficult without you. I apologize for being complicated and self-centred at times, especially during the last months. Hopefully, I can make it up to you all when I turn the page and start the next chapter in my life.

Populärvetenskaplig sammanfattning

I slutet av 1950-talet började klarsynta forskare se de stora vinster som är kopplade till att göra en del saker väldigt små, närmare bestämt mindre än en tusendels meter. Detta var mikroteknologins födelse. Förutom minskningen i volym och vikt kunde ett flertal andra fördelar förutses inom olika tillämpningsområden. Det stora genombrottet kom under 1960-talet då metoder för att producera miniatyriserad elektronik, så kallade integrerade kretsar, utvecklades. Som vi alla vet utlöste detta det paradigmskifte som lett oss in i en ny tidsepok, informationsåldern. Efterhand som forskningsfältet utvecklades utkristalliserades tre huvudgrenar; mikroelektroniken, mikromekaniken och mikrofluidiken. Eftersom samma tillverkningsmetoder används kan system från de olika grenarna enkelt integreras för att effektivt lösa komplicerade uppgifter.

Mikroelektronikindustrin omsätter idag astronomiska belopp och produkterna finns i allt från elektriska tandborstar och brödrostar till datorer och bilar. Mikroelektronik kombineras ofta med mikromekanik i komponenter som exempelvis används i videoprojektorer, mikrofoner och utlösningsmekanismer till airbags. Mikrofluidiken är den gren inom mikroteknologin som hittills haft minst genomslag men som förväntas stå inför sitt stora genombrott inom en snar framtid. Fördelarna med att hantera vätskor i mikroskala är många och stora. Till att börja med blir systemens yta stor i förhållande till volymen vilket gör att interaktionen mellan ytor och vätskor blir effektiv. Detta har stor betydelse till exempel när en yta agerar som katalysator i kemiska reaktioner eller fungerar som detektor. Vidare krävs mycket små vätskevolymer vilket innebär att kostnader kan hållas nere, prover varar längre och prover som under normala omständigheter hade varit för små för att användas kan tas till vara. Dessutom medför de korta diffusionsavstånden i systemen att kemiska reaktioner sker mycket snabbt. Möjliga tillämpningar av mikrofluidiksystem finns till exempel inom medicinsk diagnostik, molekylärbiologi, gensekvensering, kemikalieproduktion, läkemedelsframställning och proteomik. En av ledstjärnorna inom mikrofluidiken är strävan mot så kallade "lab-on-a-chip"-system eller "micro total analysis systems" (μ-TAS) där alla steg som utförs i ett normalstort laboratorium istället utförs i ett enda mikrochip. I många av de applikationer där sådana system är tänkta att användas är det centralt att kunna hantera mikropartiklar på ett effektivt sätt. Partiklarna kan exempelvis vara olika typer av celler eller artificiella partiklar med eller utan aktiva ytbeläggningar. Typiska partikelhanteringsuppgifter är att separera partiklar från andra partiklar, vätskor eller molekylära substanser. Vidare är det önskvärt att kunna styra partiklarnas väg genom nätverk av flödeskanaler, hålla fast dem i förutbestämda positioner samt att sortera dem. Bland annat dessa uppgifter kan lösas med hjälp av akustisk partikelhantering i mikroskala.

Akustisk partikelhantering bygger på en kombination av laminära flöden (flöden utan turbulens) och stående vågor (vågfenomen som resulterar i pulserande tryckfält). Mikropartiklar som befinner sig i en stående våg påverkas av akustiska krafter som flyttar dem mot antingen de delar av den stående vågen där tryckvariationerna är som störst eller de där de är som minst. Mot vilken av dessa positioner en viss partikeltyp flyttas och med vilken hastighet det sker avgörs av partiklarnas storlek, densitet och

kompressibilitet, det senare är ett mått på partiklarnas komprimerbarhet. Om en stående våg genereras i en flödeskanal kan partiklar flyttas till vissa förutsägbara positioner i flödesprofilen under tiden som de transporteras genom kanalen. Eftersom flödet är laminärt förblir fördelningen av partiklarna över flödesprofilen konstant även efter att de lämnat den del av kanalen där de påverkas av de akustiska krafterna. I nästa steg kan därför flödesprofilen delas upp med hjälp av flödesdelare på ett sådant sätt att olika partikeltyper skiljs från varandra eller från delar av den vätska de befinner sig i. I mer avancerade system kan partiklar även flyttas från en vätska till en annan och fler än två partikeltyper separeras samtidigt. Några av de huvudsakliga fördelarna med akustisk partikelhantering är att hanteringen är beröringsfri, den mekaniska belastningen på partiklarna är låg och att metoderna i många fall kan göras kontinuerliga.

I det arbete som redovisas i denna avhandling kombineras mikrofabricering, mikrofluidik och akustiska krafter för att realisera system och metoder för hantering av mikropartiklar som är lämpade för både "lab-on-a-chip"-applikationer och applikationer där kapacitetskraven är större. Detta gjordes genom att en ny konstruktionsprincip för akustiska partikelhanteringssystem, den så kallade Lundametoden, utvecklades och utvärderades experimentellt. Konstruktionen består av en kiselskiva i vilken flödeskanaler etsats och sedan tillsluts med hjälp av en glasskiva. Flödeskanalen exciteras därefter med ultraljud genom att en ultraljudsgenererande keram ansluts till kiselskivans baksida med hjälp av ultraljudsgel. En av de viktigaste fördelarna med Lundametoden är att standardmetoder för mikrofabricering används, vilket betyder att mycket hög precision kan uppnås till relativt låga kostnader samt att systemen lätt kan integreras med andra mikrosystem. Det faktum att konstruktionen dessutom är tämligen okomplicerad erbjuder stabila system och enkel kapacitetsökning genom parallellisering. Vidare är systemen speciellt lämpliga i applikationer där flödeskanalerna kontamineras eftersom den ultraljudsgenererande keramen kan frigöras och användas igen medan resten av systemet kasseras. En ytterligare fördel är att hela partikelhanteringsprocessen kan övervakas visuellt genom glaset på chipens ovansida.

De system baserade på Lundametoden som presenteras utgör en verktygslåda med metoder som kan användas var för sig eller kombineras för att lösa komplicerade partikelhanteringsuppgifter. Ett antal metoder för separation, sortering, tvätt och distribution av partiklar beskrivs och appliceras. Bland annat separeras fettpartiklar, som orsakar hjärnskador efter exempelvis bypassoperationer, och andra förorenande substanser från röda blodkroppar. Syftet är att möjliggöra ett snabbare tillfrisknande för patienterna samt att minska belastningen på blodbankerna. Ytterligare tillämpningar, inriktade på blodkomponenthantering, erbjuder nya och potentiellt effektivare alternativ inom den rutinmässiga hanteringen av blodkomponenter på blodcentralerna. Även om de undersökta applikationsområdena huvudsakligen funnits inom hantering av blodkomponenter är metoderna generiska och kan användas för att hantera i stort sett alla typer av biologiska och ickebiologiska mikropartiklar.

References

1.   Kundt, A. and O. Lehmann, *Longitudinal vibrations and acoustic figures in cylindrical columns of liquids.* Annalen der Physik und Chemie, 1874. **153**.

2.   Nilsson, J. and H. Westling, *Ultrasound in Lund - three world premieres.* Clinical Physiology and Functional Imaging, 2004. **24**(3): p. 137-140.

3.   Edler, I. and H. Hertz, *The use of the ultrasonic reflectoscope for the continuous recording of the movements of heart walls.* Kungliga Fysiografiska Sällskapets Förhandlingar, 1954. **24**(5): p. 1-19.

4.   Feynman, R.P., *There's plenty of room at the bottom (data storage).* Microelectromechanical Systems, Journal of, 1992. **1**(1): p. 60-66.

5.   Judy, J.W., *Microelectromechanical systems (MEMS): fabrication, design and applications.* Smart Materials & Structures, 2001. **10**(6): p. 1115-1134.

6.   Petersen, K.E., *Fabrication of an Integrated, Planar Silicon Ink-Jet Structure.* Ieee Transactions on Electron Devices, 1979. **26**(12): p. 1918-1920.

7.   Terry, S.C., *A gas chromatography system fabricated on a silicon wafer using integrated circuit technology* in *Electronics and Electrical Engineering.* 1975, Stanford University: Stanford.

8.   Terry, S.C., J.H. Jerman, and J.B. Angell, *Gas-Chromatographic Air Analyzer Fabricated on a Silicon-Wafer.* Ieee Transactions on Electron Devices, 1979. **26**(12): p. 1880-1886.

9.   Bassous, E., H.H. Taub, and L. Kuhn, *Ink Jet Printing Nozzle Arrays Etched in Silicon.* Applied Physics Letters, 1977. **31**(2): p. 135-137.

10.   Manz, A., D.J. Harrison, E. Verpoorte, J.C. Fettinger, A. Paulus, H. Ludi, and H.M. Widmer, *Planar chips technology for miniaturization and integration of separation techniques into monitoring systems - capillary electrophoresis on a chip.* Journal of Chromatography A, 1992. **593**(1-2): p. 253-258.

11.   Eijkel, J.C.T. and A. van den Berg, *Nanofluidics: what is it and what can we expect from it?* Microfluidics and Nanofluidics, 2005. **1**(3): p. 249-267.

12.   Manz, A., N. Graber, and H.M. Widmer, *Miniaturized Total Chemical-Analysis Systems - a Novel Concept for Chemical Sensing.* Sensors and Actuators B-Chemical, 1990. **1**(1-6): p. 244-248.

13.   Yi, C.Q., C.W. Li, S.L. Ji, and M.S. Yang, *Microfluidics technology for manipulation and analysis of biological cells.* Analytica Chimica Acta, 2006. **560**(1-2): p. 1-23.

14.   Gijs, M.A.M., *Magnetic bead handling on-chip: new opportunities for analytical applications.* Microfluidics and Nanofluidics, 2004. **1**(1): p. 22-40.

15.   Verpoorte, E., *Beads and chips: new recipes for analysis.* Lab on a Chip, 2003. **3**(4): p. 60N-68N.

16.   Wiklund, M. and H.M. Hertz, *Ultrasonic enhancement of bead-based bioaffinity assays.* Lab on a Chip, 2006. **6**(10): p. 1279-1292.

17.   Laurell, T., F. Petersson, and A. Nilsson, *Chip integrated strategies for acoustic separation and manipulation of cells and particles.* Chemical Society Reviews, 2007. **36**(3): p. 492-506.

18.   Voldman, J., M.L. Gray, and M.A. Schmidt, *Microfabrication in biology and medicine.* Annual Review of Biomedical Engineering, 1999. **1**: p. 401-425.

19.   Petersen, K.E., W.A. McMillan, G.T.A. Kovacs, M.A. Northrup, L.A. Christel, and F. Pourahmadi, *Toward next generation clinical diagnostic instruments: scaling and new processing paradigms.* Biomedical Microdevices, 1998. **1**(1): p. 71-79.

20.   Esashi, M. and T. Ono, *From MEMS to nanomachine.* Journal of Physics D-Applied Physics, 2005. **38**(13): p. R223-R230.

21.   Gates, B.D., Q.B. Xu, J.C. Love, D.B. Wolfe, and G.M. Whitesides, *Unconventional nanofabrication.* Annual Review of Materials Research, 2004. **34**: p. 339-372.

22.   Gates, B.D., Q.B. Xu, M. Stewart, D. Ryan, C.G. Willson, and G.M. Whitesides, *New approaches to nanofabrication: Molding, printing, and other techniques.* Chemical Reviews, 2005. **105**(4): p. 1171-1196.

23.   Chen, Y. and A. Pepin, *Nanofabrication: Conventional and nonconventional methods.* Electrophoresis, 2001. **22**(2): p. 187-207.

24.   Spearing, S.M., *Materials issues in microelectromechanical systems (MEMS).* Acta Materialia, 2000. **48**(1): p. 179-196.

25.   *Semiconductor sensors*, ed. S.M. Sze. 1994, New York: John Wiley & Sons.

26.   Sze, S.M., *Semiconductor devices.* 1985, New York: John Wiley & Sons.

27.   Elwenspoek, M. and H.V. Jansen, *Silicon Micromachining.* 1998, Cambridge: Cambridge University Press.

28.   Maluf, N., *An introduction to microelectromechanical systems engineering.* 2000, London: Artech House.

29.   *MEMS and Nanotechnology Clearinghouse.*   [cited 2007 March 4]; Available from: www.memsnet.org.

30.   May, G.S. and S.M. Sze, *Fundamentals of semiconductor fabrication.* 2003, New York: John Wiley & Sons.

31.   May, G.S. and C.J. Spanos, *Fundamentals of semiconductor manufacturing and process control.* 2006, New York: John Wiley & Sons.

32.   Turley, J.L., *Essential guide to semiconductor technology.* 2002, Upper Saddle River: Prentice Hall.

33.   Heule, M., S. Vuillemin, and L.J. Gauckler, *Powder-based ceramic meso- and microscale fabrication processes.* Advanced Materials, 2003. **15**(15): p. 1237-1245.

34.   McNeely, J.B., R.B. Hall, A.M. Barnett, and W.A. Tiller, *Thin-Film Silicon Crystal-Growth on Low-Cost Substrates.* Journal of Crystal Growth, 1984. **70**(1-2): p. 420-426.

35.   Bartuli, C., L. Lusvarghi, T. Manfredini, and T. Valente, *Thermal spraying to coat traditional ceramic substrates: Case studies.* Journal of the European Ceramic Society, 2007. **27**(2-3): p. 1615-1622.

36.   Roca i Cabarrocas, P., *New approaches for the production of nano-, micro-, and polycrystalline silicon thin films.* physica status solidi (c), 2004. **1**(5): p. 1115-1130.

37.   Yao, T., *Parylene fo MEMS applications*, in *Electrical Engineering.* 2002, Caltech: Pasadena.

38.   Blawas, A.S. and W.M. Reichert, *Protein patterning.* Biomaterials, 1998. **19**(7-9): p. 595-609.

39.   Rogers, J.A. and R.G. Nuzzo, *Recent progress in soft lithography.* Materials Today, 2005. **8**(2): p. 50-56.

40.   Martn, J.I., J. Nogues, K. Liu, J.L. Vicent, and I.K. Schuller, *Ordered magnetic nanostructures: fabrication and properties.* Journal of Magnetism and Magnetic Materials, 2003. **256**(1-3): p. 449-501.

41.   Arshak, K., M. Mihov, S. Nakahara, A. Arshak, and D. McDonagh, *A novel focused-ion-beam lithography process for sub-100 nanometer technology nodes.* Superlattices and Microstructures, 2004. **36**(1-3): p. 335-343.

42.   Xia, Y. and G.M. Whitesides, *Soft lithography.* Annual Review of Materials Science, 1998. **28**: p. 153-184.

43.   Laermer, F. and A. Urban, *Milestones in deep reactive ion etching.* Solid-State Sensors, Actuators and Microsystems, 2005. Digest of Technical Papers. TRANSDUCERS '05. The 13th International Conference on, 2005. **2**: p. 1118-1121.

44.   Lee, D.J., Y.H. Lee, J. Jang, and B.K. Ju, *Glass-to-glass electrostatic bonding with intermediate amorphous silicon film for vacuum packaging of microelectronics and its application.* Sensors and Actuators a-Physical, 2001. **89**(1-2): p. 43-48.

45.   Shoji, S. and M. Esashi, *Microflow Devices and Systems.* Journal of Micromechanics and Microengineering, 1994. **4**(4): p. 157-171.

46.   Ho, C.M. and Y.C. Tai, *Micro-electro-mechanical-systems (MEMS) and fluid flows.* Annual Review of Fluid Mechanics, 1998. **30**: p. 579-612.

47.   Verpoorte, E. and N.F. De Rooij, *Microfluidics meets MEMS.* Proceedings of the Ieee, 2003. **91**(6): p. 930-953.

48. Erickson, D. and D.Q. Li, *Integrated microfluidic devices.* Analytica Chimica Acta, 2004. **507**(1): p. 11-26.

49. Dittrich, P.S. and A. Manz, *Lab-on-a-chip: microfluidics in drug discovery.* Nature Reviews Drug Discovery, 2006. **5**(3): p. 210-218.

50. Nguyen, N.T. and S.T. Wereley, *Fundamentals and applications of microfluidics.* 2001, Norwood: Artech House.

51. White, F.M., *Fluid Mechanics.* 1998, New York: McGraw-Hill Education.

52. Faber, T.E., *Fluid dynamics for physicists.* 1995, Cambridge: Cambridge University Press.

53. Stone, H.A., A.D. Stroock, and A. Ajdari, *Engineering flows in small devices: Microfluidics toward a lab-on-a-chip.* Annual Review of Fluid Mechanics, 2004. **36**: p. 381-411.

54. Squires, T.M. and S.R. Quake, *Microfluidics: Fluid physics at the nanoliter scale.* Reviews of Modern Physics, 2005. **77**(3): p. 977-1026.

55. *Encyclopaedia Britannica.* [cited 2007 March 6]; Available from: www.britannica.com.

56. Nguyen, N.T. and Z.G. Wu, *Micromixers - a review.* Journal of Micromechanics and Microengineering, 2005. **15**(2): p. R1-R16.

57. Young, H.D. and R.A. Freedman, *University physics, 9th ed.* 1996, Reading: Addison-Wesley.

58. Weigl, B.H., M.R. Holl, D. Schutte, J.P. Brody, and P. Yager, *Diffusion-based optical chemical detection in silicon flow structures*, in *The International Conference on Miniaturized Systems for Chemistry and Life Sciences.* 1996. p. 174-184.

59. Kawaguchi, H., *Functional polymer microspheres.* Progress in Polymer Science, 2000. **25**(8): p. 1171-1210.

60. Meza, M.B., *Bead-based HTS applications in drug discovery.* Drug Discovery Today, 2000: p. 38-41.

61. Aulton, M.E., *Pharmaceutics.* 2001, Oxford: Harcourt Publishers.

62. Lindken, R., J. Westerweel, and B. Wieneke, *Stereoscopic micro particle image velocimetry.* Experiments in Fluids, 2006. **41**(2): p. 161-171.

63. Bitsch, L., L.H. Olesen, C.H. Westergaard, H. Bruus, H. Klank, and J.P. Kutter, *Micro particle-image velocimetry of bead suspensions and blood flows.* Experiments in Fluids, 2005. **39**(3): p. 505-511.

64. Grant, I., *Particle image velocimetry: A review.* Proceedings of the Institution of Mechanical Engineers Part C-Journal of Mechanical Engineering Science, 1997. **211**(1): p. 55-76.

65. Kinsler, L.E., A.R. Frey, and A.B. Coppens, *Fundamentals of acoustics, 4th ed.* 2000, New York: John Wiley & Sons.

66. Rienstra, S.W. and A. Hirschberg. *An introduction to acoustics.* 2004 [cited 2007 March 7]; Available from: www.win.tue.nl/%7Esjoerdr/papers/boek.pdf.

67. Cheeke, J.D.N., *Fundamentals and applications of ultrasonic waves.* 2002, Boca Raton: CRC Press.

68. Éi'piner, I.E., *Ultrasound: physical, chemical and biological effects.* 1964, New York: Consultants Bureau.

69. Berg, R.E. and D.G. Stork, *The physics of sound.* 1995, Englewood Cliffs: Prentice Hall.

70. Blitz, J., *Fundamentals of ultrasonics.* 1963, London: Butterworths.

71. Elmore, W.C. and M.A. Heald, *Physics of waves.* 1985, New York: Dover Publications.

72. Schmerr, L.W., *Fundamentals of ultrasonic nondestructive evaluation, a modeling approach.* 1998, London: Plenum Press.

73. *Ultrasound in medicine*, ed. F.A. Duck, A.C. Baker, and H.C. Starritt. 1998, London: Taylor & Francis.

74. Kremkau, F.W., *Diagnostic ultrasound: principles and instruments.* 2005, London: Elsevier Health Sciences.

75. Patten, D.R., *Fundamentals of ultrasonic plastic welding.* Machine Design, 2005. **77**(3): p. 59-61.

76. Mason, T.J., *Developments in ultrasound - Non-medical.* Progress in Biophysics & Molecular Biology, 2007. **93**(1-3): p. 166-175.

77. Mason, T.J., *Sonochemistry and sonoprocessing: the link, the trends and (probably) the future.* Ultrasonics Sonochemistry, 2003. **10**(4-5): p. 175-179.

78. Mason, T.J., E. Joyce, S.S. Phull, and J.P. Lorimer, *Potential uses of ultrasound in the biological decontamination of water.* Ultrasonics Sonochemistry, 2003. **10**(6): p. 319-323.

79. *Piezoelectric ceramics*, ed. J. van Randeraat and R.E. Setterington. 1974, London: Mullard.

80. Groschl, M., *Ultrasonic separation of suspended particles - Part I: Fundamentals.* Acustica, 1998. **84**(3): p. 432-447.

81. Chu, B.T. and R.E. Apfel, *Acoustic Radiation Pressure Produced by a Beam of Sound.* Journal of the Acoustical Society of America, 1982. **72**(6): p. 1673-1687.

82. Yosioka, K. and Y. Kawasima, *Acoustic radiation pressure on a compressible sphere.* Acustica, 1955. **5**: p. 167-173.

83. King, L.V., *On the Acoustic Radiation Pressure on Spheres.* Proceedings of the Royal Society of London. Series A, Mathematical and Physical Sciences, 1934. **147**(861): p. 212-240.

84. Gorkov, L.P., *On the forces acting on a small particle in an acoustical field in an ideal fluid.* Soviet Physics - Doklady, 1962. **6**(9): p. 773-775.

85. Kamakura, T., K. Yasuda, and Y. Kumamoto, *Unified description of second-order phenomena in sound waves.* Electronics and Communications in Japan Part Iii-Fundamental Electronic Science, 1999. **82**(2): p. 76-82.

86. Guz, A.N. and A.P. Zhuk, *Motion of solid particles in a liquid under the action of an acoustic field: The mechanism of radiation pressure.* International Applied Mechanics, 2004. **40**(3): p. 246-265.

87. Torr, G.R., *The Acoustic Radiation Force.* American Journal of Physics, 1984. **52**(5): p. 402-408.

88. Weiser, M.A.H., R.E. Apfel, and E.A. Neppiras, *Interparticle Forces on Red-Cells in a Standing Wave Field.* Acustica, 1984. **56**(2): p. 114-119.

89. Ter Haar, G. and S.J. Wyard, *Blood cell banding in ultrasonic standing wave fields: A physical analysis.* Ultrasound in Medicine and Biology, 1978. **4**(2): p. 111-123.

90. Woodside, S.M., B.D. Bowen, and J.M. Piret, *Measurement of ultrasonic forces for particle-liquid separations.* Aiche Journal, 1997. **43**(7): p. 1727-1736.

91. Nyborg, W.L., *Radiation pressure on a small rigid sphere.* Journal of the Acoustical Society of America, 1967. **42**(2): p. 947-952.

92. Gould, R.K. and W.T. Coakley, *The effects of acoustic forces on small particles in suspension*, in *Symposium on Finite Amplitude Wave Effects in Fluids* 1973. p. 252-257.

93. Yasuda, K. and T. Kamakura, *Acoustic radiation force on micrometer-size particles.* Applied Physics Letters, 1997. **71**(13): p. 1771-1773.

94. Spengler, J.F., W.T. Coakley, and K.T. Christensen, *Microstreaming effects on particle concentration in an ultrasonic standing wave.* Aiche Journal, 2003. **49**(11): p. 2773-2782.

95. Woodside, S.M., J.M. Piret, M. Groschl, E. Benes, and B.D. Bowen, *Acoustic force distribution in resonators for ultrasonic particle separation.* Aiche Journal, 1998. **44**(9): p. 1976-1984.

96. Masudo, T. and T. Okada, *Particle characterization and separation by a coupled acoustic-gravity field.* Analytical Chemistry, 2001. **73**(14): p. 3467-3471.

97. Toubal, M., M. Asmani, E. Radziszewski, and B. Nongaillard, *Acoustic measurement of compressibility and thermal expansion coefficient of erythrocytes.* Physics in Medicine and Biology, 1999. **44**(5): p. 1277-1287.

98. Whitworth, G., M.A. Grundy, and W.T. Coakley, *Transport and Harvesting of Suspended Particles Using Modulated Ultrasound.* Ultrasonics, 1991. **29**(6): p. 439-444.

99. Tuziuti, T., T. Kozuka, and H. Mitome, *Measurement of distribution of acoustic radiation force perpendicular to sound beam axis.* Japanese Journal of Applied Physics Part 1-Regular Papers Short Notes & Review Papers, 1999. **38**(5B): p. 3297-3301.

100. König, W., *Hydrodynamisch-akustische Untersuchungen: II. Über die Kräfte zwischen zwei Kugeln in einer schwingenden Flüssigkeit und über die Entstehung der Kundt'schen Staubfiguren* Annalen der Physik und Chemie, 1891. **42**(4): p. 549-563.

101. Bjerknes, V.F.K., *Die Kraftfelder.* 1909, Braunschweig: Vieweg und Sohn.

102. Crum, L.A., *Bjerknes Forces on Bubbles in a Stationary Sound Field.* Journal of the Acoustical Society of America, 1975. **57**(6): p. 1363-1370.

103. Zheng, X.Y. and R.E. Apfel, *Acoustic Interaction Forces between 2 Fluid Spheres in an Acoustic Field.* Journal of the Acoustical Society of America, 1995. **97**(4): p. 2218-2226.

104. Doinikov, A.A., *Acoustic radiation interparticle forces in a compressible fluid.* Journal of Fluid Mechanics, 2001. **444**: p. 1-21.

105. Ida, M., *Alternative interpretation of the sign reversal of secondary Bjerknes force acting between two pulsating gas bubbles.* Physical Review E, 2003. **67**(5).

106. Pelekasis, N.A. and J.A. Tsamopoulos, *Bjerknes Forces between 2 Bubbles .1. Response to a Step Change in Pressure.* Journal of Fluid Mechanics, 1993. **254**: p. 467-499.

107. Pelekasis, N.A. and J.A. Tsamopoulos, *Bjerknes Forces between 2 Bubbles .2. Response to an Oscillatory Pressure Field.* Journal of Fluid Mechanics, 1993. **254**: p. 501-527.

108. Spengler, J.F. and W.T. Coakley, *Ultrasonic trap to monitor morphology and stability of developing microparticle aggregates.* Langmuir, 2003. **19**(9): p. 3635-3642.

109. Spengler, J.F., M. Jekel, K.T. Christensen, R.J. Adrian, J.J. Hawkes, and W.T. Coakley, *Observation of yeast cell movement and aggregation in a small-scale MHz-ultrasonic standing wave field.* Bioseparation, 2000. **9**(6): p. 329-341.

110. Strutt, J.W., *On the circulation of air observed in Kundt's tube and on some allied acoustical problems.* Phil. Trans. R. Soc. London, 1883. **175**: p. 1.

111. Strutt, J.W., *The theory of sound.* 1896, New York: Dover Publications.

112. Hamilton, M.F., Y.A. Ilinskii, and E.A. Zabolotskaya, *Thermal effects on acoustic streaming in standing waves.* Journal of the Acoustical Society of America, 2003. **114**(6): p. 3092-3101.

113. Hamilton, M.F., Y.A. Ilinskii, and E.A. Zabolotskaya, *Acoustic streaming generated by standing waves in two-dimensional channels of arbitrary width.* Journal of the Acoustical Society of America, 2003. **113**(1): p. 153-160.

114. Bengtsson, M. and T. Laurell, *Ultrasonic agitation in microchannels.* Analytical and Bioanalytical Chemistry, 2004. **378**(7): p. 1716-1721.

115. Lighthill, J., *Acoustic Streaming.* Journal of Sound and Vibration, 1978. **61**(3): p. 391-418.

116. Riley, N., *Acoustic streaming.* Theoretical and Computational Fluid Dynamics, 1998. **10**(1-4): p. 349-356.

117. Elder, S.A., *Cavitation Microstreaming.* Journal of the Acoustical Society of America, 1959. **31**(1): p. 54-64.

118. Giddings, J.C., *Unified separation science.* 1991, New York: John Wiley & Sons.

119. Pamme, N., *Magnetism and microfluidics.* Lab on a Chip, 2006. **6**(1): p. 24-38.

120. van Hee, P., M.A. Hoeben, R. van der Lans, and L.A.M. van der Wielen, *Strategy for selection of methods for separation of bioparticles from particle mixtures.* Biotechnology and Bioengineering, 2006. **94**(4): p. 689-709.

121. Friedman, G. and B. Yellen, *Magnetic separation, manipulation and assembly of solid phase in fluids.* Current Opinion in Colloid & Interface Science, 2005. **10**(3-4): p. 158-166.

122. Han, K.H. and A.B. Frazier, *Paramagnetic capture mode magnetophoretic microseparator for high efficiency blood cell separations.* Lab on a Chip, 2006. **6**(2): p. 265-273.

123. Watarai, H., M. Suwa, and Y. Iiguni, *Magnetophoresis and electromagnetophoresis of microparticles in liquids.* Analytical and Bioanalytical Chemistry, 2004. **378**: p. 1693-1699.

124. Pamme, N. and A. Manz, *On-chip free-flow magnetophoresis: Continuous flow separation of magnetic particles and agglomerates.* Analytical Chemistry, 2004. **76**(24): p. 7250-7256.

125. Pamme, N. and C. Wilhelm, *Continuous sorting of magnetic cells via on-chip free-flow magnetophoresis.* Lab on a Chip, 2006. **6**: p. 974-980.

126. Kelly, R.T. and A.T. Woolley, *Electric field gradient focusing.* Journal of Separation Science, 2005. **28**(15): p. 1985-1993.

127. Serwer, P. and G.A. Griess, *Advances in the separation of bacteriophages and related particles.* Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences, 1999. **722**(1-2): p. 179-190.

128. Breadmore, M.C., *Recent advances in enhancing the sensitivity of electrophoresis and electrochromatography in capillaries and microchips.* Electrophoresis, 2007. **28**(1-2): p. 254-281.

129. Eeltink, S., G.R. Rozing, and W.T. Kok, *Recent applications in capillary electrochromatography.* Electrophoresis, 2003. **24**(22-23): p. 3935-3961.

130. Kremser, L., D. Blaas, and E. Kenndler, *Capillary electrophoresis of biological particles: Viruses, bacteria, and eukaryotic cells.* Electrophoresis, 2004. **25**(14): p. 2282-2291.

131. Zhang, C.X. and A. Manz, *High-speed free-flow electrophoresis on chip.* Analytical Chemistry, 2003. **75**(21): p. 5759-5766.

132. Raymond, D.E., A. Manz, and H.M. Widmer, *Continuous Sample Preparation Using Free-flow electrophoresis On A Silicon Microstructure.* Solid-State Sensors and Actuators, 1995 and Eurosensors IX. Transducers '95. The 8th International Conference on, 1995. **1**: p. 760-763.

133. de Jesus, D.P., L. Blanes, and C.L. do Lago, *Microchip free-flow electrophoresis on glass substrate using laser-printing toner as structural material.* Electrophoresis, 2006. **27**(24): p. 4935-4942.

134. Fonslow, B.R. and M.T. Bowser, *Free-flow electrophoresis on an anodic bonded glass microchip.* Analytical Chemistry, 2005. **77**(17): p. 5706-5710.

135. Muller, T., G. Gradl, S. Howitz, S. Shirley, T. Schnelle, and G. Fuhr, *A 3-D microelectrode system for handling and caging single cells and particles.* Biosensors & Bioelectronics, 1999. **14**(3): p. 247-256.

136. Schnelle, T., T. Muller, G. Gradl, S.G. Shirley, and G. Fuhr, *Dielectrophoretic manipulation of suspended submicron particles.* Electrophoresis, 2000. **21**(1): p. 66-73.

137. Gascoyne, P.R.C. and J. Vykoukal, *Particle separation by dielectrophoresis.* Electrophoresis, 2002. **23**(13): p. 1973-1983.

138. Hughes, M.P., *Strategies for dielectrophoretic separation in laboratory-on-a-chip systems.* Electrophoresis, 2002. **23**(16): p. 2569-2582.

139. Pertoft, H., *Fractionation of cells and subcellular particles with Percoll.* Journal of Biochemical and Biophysical Methods, 2000. **44**(1-2): p. 1-30.

140. *Guide to the preparation, use and quality assurance of blood components.* 2004, Strasbourg: Council of Europe Publishing.

141. Ito, Y. and K. Shinomiya, *A new continuous-flow cell separation method based on cell density: Principle, apparatus, and preliminary application to separation of human buffy coat.* Journal of Clinical Apheresis, 2001. **16**(4): p. 186-191.

142. Schaflinger, U., *Centrifugal Separation of a Mixture.* Fluid Dynamics Research, 1990. **6**(5-6): p. 213-249.

143. Runck, A.H. and C.R. Valeri, *Continuous-Flow Centrifugation Washing of Red Blood-Cells.* Transfusion, 1972. **12**(4): p. 237-244.

144. Stasyk, T. and L.A. Huber, *Zooming in: Fractionation strategies in proteomics.* Proteomics, 2004. **4**(12): p. 3704-3716.

145. Woodside, S.M., B.D. Bowen, and J.M. Piret, *Mammalian cell retention devices for stirred perfusion bioreactors.* Cytotechnology, 1998. **28**(1-3): p. 163-175.

146. Sethu, P., A. Sin, and M. Toner, *Microfluidic diffusive filter for apheresis (leukapheresis).* Lab on a Chip, 2006. **6**(1): p. 83-89.

147. Miller, J.M., *Chromatography, concepts and contrasts, 2nd ed.* 2004, New York: John Wiley & Sons.

148. *Field flow fractionation handbook*, ed. M.E. Schimpf, K. Caldwell, and J.C. Giddings. 2000, New York: John Wiley & Sons.

149. Edwards, T.L., B.K. Gale, and A.B. Frazier, *A microfabricated thermal field-flow fractionation system.* Analytical Chemistry, 2002. **74**(6): p. 1211-1216.

150. Giddings, J.C., *Field-Flow Fractionation - Analysis of Macromolecular, Colloidal, and Particulate Materials.* Science, 1993. **260**(5113): p. 1456-1465.

151. Reschiglian, P., A. Zattoni, B. Roda, E. Michelini, and A. Roda, *Field-flow fractionation and biotechnology.* Trends in Biotechnology, 2005. **23**(9): p. 475-483.

152. Semyonov, S.N. and K.I. Maslow, *Acoustic Field-Flow Fractionation.* Journal of Chromatography, 1988. **446**: p. 151-156.

153. Edwards, T.L. and A.B. Frazier, *A micro acoustic field-flow fractionation system for nano-scale separations*, in *The International Conference on Miniaturized Systems for Chemistry and Life Sciences.* 2004. p. 174-176.

154. Bor, C., *Split-Flow Thin Fractionation. SPLITT fractionation is a new family of separation techniques for macromolecules, colloids, and particles.* Analytical chemistry, 2000. **72**(7): p. 266 A-271 A.

155. Inglis, D.W., J.A. Davis, R.H. Austin, and J.C. Sturm, *Critical particle size for fractionation by deterministic lateral displacement.* Lab on a Chip, 2006. **6**(5): p. 655-658.

156. Huang, L.R., E.C. Cox, R.H. Austin, and J.C. Sturm, *Continuous particle separation through deterministic lateral displacement.* Science, 2004. **304**(5673): p. 987-990.

157. Davis, J.A., D.W. Inglis, K.J. Morton, D.A. Lawrence, L.R. Huang, S.Y. Chou, J.C. Sturm, and R.H. Austin, *Deterministic hydrodynamics: Taking blood apart.* Proceedings of the National Academy of Sciences of the United States of America, 2006. **103**(40): p. 14779-14784.

158. Yamada, M., M. Nakashima, and M. Seki, *Pinched flow fractionation: Continuous size separation of particles utilizing a laminar flow profile in a pinched microchannel.* Analytical Chemistry, 2004. **76**(18): p. 5465-5471.

159. Takagi, J., M. Yamada, M. Yasuda, and M. Seki, *Continuous particle separation in a microchannel having asymmetrically arranged multiple branches.* Lab on a Chip, 2005. **5**(7): p. 778-784.

160. Yamada, M. and M. Seki, *Microfluidic particle sorter employing flow splitting and recombining.* Analytical Chemistry, 2006. **78**(4): p. 1357-1362.

161. Desai, T.A., D.J. Hansford, and M. Ferrari, *Micromachined interfaces: new approaches in cell immunoisolation and biomolecular separation.* Biomolecular Engineering, 2000. **17**(1): p. 23-36.

162. Richardson, P.J. and J.P. Luzio, *Immunoaffinity Purification of Subcellular Particles and Organelles.* Applied Biochemistry and Biotechnology, 1986. **13**(2): p. 133-145.

163. MacDonald, M.P., G.C. Spalding, and K. Dholakia, *Microfluidic sorting in an optical lattice.* Nature, 2003. **426**(6965): p. 421-424.

164. *Subcellular fractionation: a practical approach*, ed. J.M. Graham and D. Rickwood. 1997, Oxford: Oxford University Press.

165. Dittrich, P.S., K. Tachikawa, and A. Manz, *Micro total analysis systems. Latest advancements and trends.* Analytical Chemistry, 2006. **78**(12): p. 3887-3907.

166. Cui, H.C., K. Horiuchi, P. Dutta, and C.F. Ivory, *Multistage isoelectric focusing in a polymeric microfluidic chip.* Analytical Chemistry, 2005. **77**(24): p. 7878-7886.

167. Cui, H.C., K. Horiuchi, P. Dutta, and C.F. Ivory, *Isoelectric focusing in a poly(dimethylsiloxane) microfluidic chip.* Analytical Chemistry, 2005. **77**(5): p. 1303-1309.

168. Yasuda, K., S. Umemura, and K. Takeda, *Particle separation using acoustic radiation force and electrostatic force.* Journal of the Acoustical Society of America, 1996. **99**(4): p. 1965-1970.

169. Hawkes, J.J., M. Groeschl, E. Benes, H. Nowotny, and W.T. Coakley, *Positioning particles within liquids using ultrasound force fields*, in *Forum Acusticum*. 2002.

170. Nowotny, H. and E. Benes, *General One-Dimensional Treatment of the Layered Piezoelectric Resonator with 2 Electrodes*. Journal of the Acoustical Society of America, 1987. **82**(2): p. 513-521.

171. Nowotny, H., E. Benes, and M. Schmid, *Layered Piezoelectric Resonators with an Arbitrary Number of Electrodes (General One-Dimensional Treatment)*. Journal of the Acoustical Society of America, 1991. **90**(3): p. 1238-1245.

172. Hawkes, J.J., W.T. Coakley, M. Groschl, E. Benes, S. Armstrong, P.J. Tasker, and H. Nowotny, *Single half-wavelength ultrasonic particle filter: Predictions of the transfer matrix multilayer resonator model and experimental filtration results*. Journal of the Acoustical Society of America, 2002. **111**(3): p. 1259-1266.

173. Hill, M., *The selection of layer thicknesses to control acoustic radiation force profiles in layered resonators*. Journal of the Acoustical Society of America, 2003. **114**(5): p. 2654-2661.

174. Hill, M., Y.J. Shen, and J.J. Hawkes, *Modelling of layered resonators for ultrasonic separation*. Ultrasonics, 2002. **40**(1-8): p. 385-392.

175. Hill, M. and R.J.K. Wood, *Modelling in the design of a flow-through ultrasonic separator*. Ultrasonics, 2000. **38**(1-8): p. 662-665.

176. Wiklund, M., *Ultrasonic enrichment of microparticles in bioaffinity assays*, in *Physics*. 2004, Royal Institute of Technology: Stockholm.

177. Nilsson, A., F. Petersson, H. Jonsson, and T. Laurell, *Acoustic control of suspended particles in micro fluidic chips*. Lab on a Chip, 2004. **4**(2): p. 131-135.

178. Nilsson, A., F. Petersson, H.W. Persson, H. Jonsson, and T. Laurell, *Manipulation of suspended particles in a laminar flow*, in *The International Conference on Miniaturized Systems for Chemistry and Life Sciences*. 2002. p. 751-753.

179. Chinnery, P.A., V.F. Humphrey, and C. Beckett, *The schlieren image of two-dimensional ultrasonic fields and cavity resonances*. Journal of the Acoustical Society of America, 1997. **101**(1): p. 250-256.

180. Neild, A., S. Oberti, and J. Dual, *Design, modeling and characterization of microfluidic devices for ultrasonic manipulation*. Sensors and Actuators B-Chemical, 2007. **121**(2): p. 452-461.

181. Townsend, R.J., M. Hill, N.R. Harris, and N.M. White, *Modelling of particle paths passing through an ultrasonic standing wave*. Ultrasonics, 2004. **42**(1-9): p. 319-324.

182. Mandralis, Z.I. and D.L. Feke, *Fractionation of Suspensions Using Synchronized Ultrasonic and Flow-Fields*. Aiche Journal, 1993. **39**(2): p. 197-206.

183. Johnson, D.A. and D.L. Feke, *Methodology for fractionating suspended particles using ultrasonic standing wave and divided flow fields*. Separations Technology, 1995. **5**(4): p. 251-258.

184. Gupta, S., D.L. Feke, and I. Manaszloczower, *Fractionation of Mixed Particulate Solids According to Compressibility Using Ultrasonic Standing-Wave Fields*. Chemical Engineering Science, 1995. **50**(20): p. 3275-3284.

185. Hatanaka, S., T. Taki, M. Kuwabara, M. Sano, and S. Asai, *Effect of process parameters on ultrasonic separation of dispersed particles in liquid*. Japanese Journal of Applied Physics Part 1-Regular Papers Short Notes & Review Papers, 1999. **38**(5B): p. 3096-3100.

186. Kapishnikov, S., V. Kantsler, and V. Steinberg, *Continuous particle size separation and size sorting using ultrasound in a microchannel*. Journal of Statistical Mechanics-Theory and Experiment, 2006.

187. Santesson, S. and S. Nilsson, *Airborne chemistry: acoustic levitation in chemical analysis*. Analytical and Bioanalytical Chemistry, 2004. **378**(7): p. 1704-1709.

188. Kaduchak, G., D.N. Sinha, and D.C. Lizon, *Novel cylindrical, air-coupled acoustic levitation/concentration devices*. Review of Scientific Instruments, 2002. **73**(3): p. 1332-1336.

189. Strauss, S., *Look ma, no hands*. Technology Review, 1988. **91**(6): p. 11-13.

190. Hertz, H.M., *Standing-wave acoustic trap for nonintrusive positioning of microparticles.* Journal of Applied Physics, 1995. **78**(8): p. 4845-4849.

191. Limaye, M.S., J.J. Hawkes, and W.T. Coakley, *Ultrasonic standing wave removal of microorganisms from suspension in small batch systems.* Journal of Microbiological Methods, 1996. **27**(2-3): p. 211-220.

192. Limaye, M.S. and W.T. Coakley, *Clarification of small volume microbial suspensions in an ultrasonic standing wave.* Journal of Applied Microbiology, 1998. **84**(6): p. 1035-1042.

193. Cousins, C.M., P. Holownia, J.J. Hawkes, M.S. Limaye, C.P. Price, P.J. Keay, and W.T. Coakley, *Plasma preparation from whole blood using ultrasound.* Ultrasound in Medicine and Biology, 2000. **26**(5): p. 881-888.

194. Cousins, C.M., J.R. Melin, W.A. Venables, and W.T. Coakley, *Investigation of enhancement of two processes, sedimentation and conjugation, when bacteria are concentrated in ultrasonic standing waves.* Bioseparation, 2000. **9**(6): p. 343-349.

195. Wiklund, M., J. Toivonen, M. Tirri, P. Hanninen, and H.M. Hertz, *Ultrasonic enrichment of microspheres for ultrasensitive biomedical analysis in confocal laser-scanning fluorescence detection.* Journal of Applied Physics, 2004. **96**(2): p. 1242-1248.

196. Pui, P.W.S., F. Trampler, S.A. Sonderhoff, M. Groeschl, D.G. Kilburn, and J.M. Piret, *Batch and Semicontinuous Aggregation and Sedimentation of Hybridoma Cells by Acoustic-Resonance Fields.* Biotechnology Progress, 1995. **11**(2): p. 146-152.

197. Coakley, W.T., *Ultrasonic separations in analytical biotechnology.* Trends in Biotechnology, 1997. **15**(12): p. 506-511.

198. Coakley, W.T., J.J. Hawkes, M.A. Sobanski, C.M. Cousins, and J. Spengler, *Analytical scale ultrasonic standing wave manipulation of cells and microparticles.* Ultrasonics, 2000. **38**(1-8): p. 638-641.

199. Groschl, H., W. Burger, and B. Handl, *Ultrasonic separation of suspended particles - Part III: Application in biotechnology.* Acustica, 1998. **84**(5): p. 815-822.

200. Trampler, F., S.A. Sonderhoff, P.W.S. Pui, D.G. Kilburn, and J.M. Piret, *Acoustic Cell Filter for High-Density Perfusion Culture of Hybridoma Cells.* Bio-Technology, 1994. **12**(3): p. 281-284.

201. Gaida, T., O. DoblhoffDier, K. Strutzenberger, H. Katinger, W. Burger, M. Groschl, B. Handl, and E. Benes, *Selective retention of viable cells in ultrasonic resonance field devices.* Biotechnology Progress, 1996. **12**(1): p. 73-76.

202. Peterson, S., G. Perkins, and C. Baker, *Development of an ultrasonic blood cell separator*, in *IEEE / Annual Conference of the Engineering in Medicine and Biology Society* 1986. p. 154-156.

203. Hawkes, J.J. and W.T. Coakley, *A continuous flow ultrasonic cell-filtering method.* Enzyme and Microbial Technology, 1996. **19**(1): p. 57-62.

204. Tolt, T.L. and D.L. Feke, *Separation of Dispersed Phases from Liquids in Acoustically Driven Chambers.* Chemical Engineering Science, 1993. **48**(3): p. 527-540.

205. Benes, E., M. Groschl, H. Nowotny, F. Trampler, T. Keijzer, H. Bohm, S. Radel, L. Gherardini, J.J. Hawkes, R. Konig, and C. Delouvroy, *Ultrasonic separation of suspended particles.* Ultrasonics Symposium, 2001 IEEE, 2001. **1**: p. 649-659 vol.1.

206. Bohm, H., L.G. Briarty, K.C. Lowe, J.B. Power, E. Benes, and M.R. Davey, *Quantification of a novel h-shaped ultrasonic resonator for separation of biomaterials under terrestrial gravity and microgravity conditions.* Biotechnology and Bioengineering, 2003. **82**(1): p. 74-85.

207. Groschl, M., *Ultrasonic separation of suspended particles - Part II: Design and operation of separation devices.* Acustica, 1998. **84**(4): p. 632-642.

208. Hawkes, J.J. and W.T. Coakley, *Force field particle filter, combining ultrasound standing waves and laminar flow.* Sensors and Actuators B-Chemical, 2001. **75**(3): p. 213-222.

209. Yasuda, K., S. Umemura, and K. Takeda, *Concentration and Fractionation of Small Particles in Liquid by Ultrasound.* Japanese Journal of Applied Physics Part 1-Regular Papers Short Notes & Review Papers, 1995. **34**(5B): p. 2715-2720.

210. Kumar, M., D.L. Feke, and J.M. Belovich, *Fractionation of cell mixtures using acoustic and laminar flow fields.* Biotechnology and Bioengineering, 2005. **89**(2): p. 129-137.

211. Mandralis, Z.I. and D.L. Feke, *Continuous Suspension Fractionation Using Acoustic and Divided-Flow Fields.* Chemical Engineering Science, 1993. **48**(23): p. 3897-3905.

212. Hill, M., J.J. Hawkes, N.R. Harris, and M.B. McDonnell, *Resonant ultrasonic particle manipulators and their applications in sensor systems.* Sensors, 2004. Proceedings of IEEE, 2004: p. 794-797.

213. Masudo, T. and T. Okada, *Particle separation with ultrasound radiation force.* Current Analytical Chemistry, 2006. **2**(2): p. 213-227.

214. Gupta, S. and D.L. Feke, *Filtration of particulate suspensions in acoustically driven porous media.* Aiche Journal, 1998. **44**(5): p. 1005-1014.

215. Gupta, S. and D.L. Feke, *Acoustically driven collection of suspended particles within porous media.* Ultrasonics, 1997. **35**(2): p. 131-139.

216. Grossner, M.T., A.E. Penrod, J.M. Belovich, and D.L. Feke, *Single fiber model of particle retention in an acoustically driven porous mesh.* Ultrasonics, 2003. **41**(2): p. 65-74.

217. Pangu, G.D. and D.L. Feke, *Acoustically aided separation of oil droplets from aqueous emulsions.* Chemical Engineering Science, 2004. **59**(15): p. 3183-3193.

218. Mandralis, Z.I., W. Bolek, W. Burger, E. Benes, and D.L. Feke, *Enhanced synchronized ultrasonic and flow-field fractionation of suspensions.* Ultrasonics, 1994. **32**(2): p. 113-121.

219. Wiklund, M., P. Spegel, S. Nilsson, and H.M. Hertz, *Ultrasonic-trap-enhanced selectivity in capillary electrophoresis.* Ultrasonics, 2003. **41**(4): p. 329-333.

220. Kuznetsova, L.A., S.P. Martin, and W.T. Coakley, *Sub-micron particle behaviour and capture at an immuno-sensor surface in an ultrasonic standing wave.* Biosensors & Bioelectronics, 2005. **21**(6): p. 940-948.

221. Martin, S.P., R.J. Townsend, L.A. Kuznetsova, K.A.J. Borthwick, M. Hill, M.B. McDonnell, and W.T. Coakley, *Spore and micro-particle capture on an immunosensor surface in an ultrasound standing wave system.* Biosensors & Bioelectronics, 2005. **21**(5): p. 758-767.

222. Kozuka, T., T. Tuziuti, H. Mitome, T. Fukuda, and F. Arai, *Two-dimensional acoustic micromanipulation using three ultrasonic transducers.* Micromechatronics and Human Science, 1998. MHS '98. Proceedings of the 1998 International Symposium on, 1998: p. 201-204.

223. Hawkes, J.J., M.J. Long, W.T. Coakley, and M.B. McDonnell, *Ultrasonic deposition of cells on a surface.* Biosensors & Bioelectronics, 2004. **19**(9): p. 1021-1028.

224. Hawkes, J.J., R.W. Barber, D.R. Emerson, and W.T. Coakley, *Continuous cell washing and mixing driven by an ultrasound standing wave within a microfluidic channel.* Lab on a Chip, 2004. **4**(5): p. 446-452.

225. Harris, N., M. Hill, Y. Shen, R.J. Townsend, S. Beeby, and N. White, *A dual frequency, ultrasonic, microengineered particle manipulator.* Ultrasonics, 2004. **42**(1-9): p. 139-144.

226. Harris, N.R., M. Hill, S. Beeby, Y. Shen, N.M. White, J.J. Hawkes, and W.T. Coakley, *A silicon microfluidic ultrasonic separator.* Sensors and Actuators B-Chemical, 2003. **95**(1-3): p. 425-434.

227. Lilliehorn, T., M. Nilsson, U. Simu, S. Johansson, M. Almqvist, J. Nilsson, and T. Laurell, *Dynamic arraying of microbeads for bioassays in microfluidic channels.* Sensors and Actuators B-Chemical, 2005. **106**(2): p. 851-858.

228. Lilliehorn, T., U. Simu, M. Nilsson, M. Almqvist, T. Stepinski, T. Laurell, J. Nilsson, and S. Johansson, *Trapping of microparticles in the near field of an ultrasonic transducer.* Ultrasonics, 2005. **43**(5): p. 293-303.

229. Haake, A., A. Neild, D.H. Kim, J.E. Ihm, Y. Sun, J. Dual, and B.K. Ju, *Manipulation of cells using an ultrasonic pressure field.* Ultrasound in Medicine and Biology, 2005. **31**(6): p. 857-864.

230. Neild, A., S. Oberti, F. Beyeler, J. Dual, and B.J. Nelson, *A micro-particle positioning technique combining an ultrasonic manipulator and a microgripper.* Journal of Micromechanics and Microengineering, 2006. **16**(8): p. 1562-1570.

231. Evander, M., L. Johansson, T. Lilliehorn, J. Piskur, M. Lindvall, S. Johansson, M. Almqvist, T. Laurell, and J. Nilsson, *Noninvasive Acoustic Cell Trapping in a Microfluidic Perfusion System for Online Bioassays.* Analytical Chemistry, 2007. **79**(7): p. 2984-2991.

232. Wiklund, M., C. Gunther, R. Lemor, M. Jager, G. Fuhr, and H.M. Hertz, *Ultrasonic standing wave manipulation technology integrated into a dielectrophoretic chip.* Lab on a Chip, 2006. **6**(12): p. 1537-1544.

233. Nyborg, W.L., *Safety of medical diagnostic ultrasound.* Seminars in Ultrasound Ct and Mri, 2002. **23**(5): p. 377-386.

234. Dalecki, D., *Mechanical bioeffects of ultrasound.* Annual Review of Biomedical Engineering, 2004. **6**: p. 229-248.

235. Borthwick, K.A.J., W.T. Coakley, M.B. McDonnell, H. Nowotny, E. Benes, and M. Groschl, *Development of a novel compact sonicator for cell disruption.* Journal of Microbiological Methods, 2005. **60**(2): p. 207-216.

236. Apfel, R.E., *Sonic effervescence: A tutorial on acoustic cavitation.* Journal of the Acoustical Society of America, 1997. **101**(3): p. 1227-1237.

237. Bohm, H., P. Anthony, M.R. Davey, L.G. Briarty, J.B. Power, K.C. Lowe, E. Benes, and M. Groschl, *Viability of plant cell suspensions exposed to homogeneous ultrasonic fields of different energy density and wave type.* Ultrasonics, 2000. **38**(1-8): p. 629-632.

238. Yasuda, K., S.S. Haupt, S. Umemura, T. Yagi, M. Nishida, and Y. Shibata, *Using acoustic radiation force as a concentration method for erythrocytes.* Journal of the Acoustical Society of America, 1997. **102**(1): p. 642-645.

239. Yasuda, K., *Non-destructive, non-contact handling method for biomaterials in micro-chamber by ultrasound.* Sensors and Actuators B-Chemical, 2000. **64**(1-3): p. 128-135.

240. Daniels, S., T. Kodama, and D.J. Price, *Damage to Red-Blood-Cells Induced by Acoustic Cavitation.* Ultrasound in Medicine and Biology, 1995. **21**(1): p. 105-111.

241. Howkins, S.D. and Weinstoc.A, *Effect of Focused Ultrasound on Human Blood.* Ultrasonics, 1970. **8**(3): p. 174-&.

242. Jonsson, H., C. Holm, A. Nilsson, F. Petersson, P. Johnsson, and T. Laurell, *Particle separation using ultrasound can radically reduce embolic load to brain after cardiac surgery.* Annals of Thoracic Surgery, 2004. **78**(5): p. 1572-1578.

243. Hultstrom, J., O. Manneberg, K. Dopf, H.M. Hertz, H. Brismar, and M. Wiklund, *Proliferation and viability of adherent cells manipulated by standing-wave ultrasound in a microfluidic chip.* Ultrasound in Medicine and Biology, 2007. **33**(1): p. 145-151.

244. Bazou, D., L.A. Kuznetsova, and W.T. Coakley, *Physical enviroment of 2-D animal cell aggregates formed in a short pathlength ultrasound standing wave trap.* Ultrasound in Medicine and Biology, 2005. **31**(3): p. 423-430.

245. Radel, S., A.J. McLoughlin, L. Gherardini, O. Doblhoff-Dier, and E. Benes, *Viability of yeast cells in well controlled propagating and standing ultrasonic plane waves.* Ultrasonics, 2000. **38**(1-8): p. 633-637.

246. Wang, Z.W., P. Grabenstetter, D.L. Feke, and J.M. Belovich, *Retention and viability characteristics of mammalian cells in an acoustically driven polymer mesh.* Biotechnology Progress, 2004. **20**(1): p. 384-387.

247. Petersson, F., A. Nilsson, C. Holm, H. Jonsson, and T. Laurell, *Separation of lipids from blood utilizing ultrasonic standing waves in microfluidic channels.* Analyst, 2004. **129**(10): p. 938-943.

248. Petersson, F., A. Nilsson, C. Holm, H. Jonsson, and T. Laurell, *Continuous separation of lipid particles from erythrocytes by means of laminar flow and acoustic standing wave forces.* Lab on a Chip, 2005. **5**(1): p. 20-22.

249. Nilsson, A., F. Petersson, and T. Laurell, *Whole blood plasmapheresis using acoustic separation chips*, in *The International Conference on Miniaturized Systems for Chemistry and Life Sciences*. 2006. p. 314-316.

250. Nilsson, A., F. Petersson, H.W. Persson, H. Jonsson, and T. Laurell, *Autologous blood recovery and wash in microfluidic channel arrays utilizing ultrasonic standing waves*, in *The International Conference on Miniaturized Systems for Chemistry and Life Sciences*. 2002. p. 625-626.

251. Jonsson, H., A. Nilsson, F. Petersson, M. Allers, and T. Laurell, *Particle separation using ultrasound can be used with human shed mediastinal blood*. Perfusion-Uk, 2005. **20**(1): p. 39-43.

252. Petersson, F., A. Nilsson, H. Jonsson, and T. Laurell, *Particle flow switch utilizing ultrasonic particle switching in microfluidic channels*, in *The International Conference on Miniaturized Systems for Chemistry and Life Sciences*. 2003. p. 879-882.

253. Petersson, F., A. Nilsson, H. Jonsson, and T. Laurell, *Carrier medium exchange through ultrasonic particle switching in microfluidic channels*. Analytical Chemistry, 2005. **77**(5): p. 1216-1221.

254. Petersson, F., L.B. Åberg, A.K. Swärd-Nilsson, and T. Laurell, *Free flow acoustophoresis (FFA) - a new microfluidic based mode of particle and cell separtion (manuscript)*. 2007.

255. Petersson, F., L.B. Åberg, and T. Laurell, *Acoustic separation of particles with similar acoustic properties by means of medium density manipulation*, in *The International Conference on Miniaturized Systems for Chemistry and Life Sciences*. 2006. p. 540-542.

256. Sundin, M., A. Nilsson, F. Petersson, and T. Laurell, *Binary valving of particles using acoustic forces*, in *The International Conference on Miniaturized Systems for Chemistry and Life Sciences*. 2004. p. 662-664.

257. Siversson, C., F. Petersson, A. Nilsson, and T. Laurell, *Acoustic particle sizing in microchannels by means of ultrasonic frequency switching*, in *The International Conference on Miniaturized Systems for Chemistry and Life Sciences*. 2004. p. 330-332.

258. Augustsson, P., F. Petersson, and T. Laurell, *Improved carrier medium exchange efficiency in acoustic standing wave particle washing*, in *The International Conference on Miniaturized Systems for Chemistry and Life Sciences*. 2006. p. 627-629.

259. Petersson, F. and T. Laurell, *Acoustic sorting of particles in microfluidic channels*, in *International symposium on field-flow fractionation*. 2005.

260. Engstrom, K.G., *Contaminating fat in pericardial suction blood: a clinical, technical and scientific challenge*. Perfusion-Uk, 2004. **19**: p. S21-S31.

261. Solis, R.T., G.P. Noon, A.C. Beall, and M.E. Debakey, *Particulate Microembolism During Cardiac Operation*. Annals of Thoracic Surgery, 1974. **17**(4): p. 332-344.

262. Liu, J.F., Z.K. Su, and W.X. Ding, *Quantitation of Particulate Microemboli During Cardiopulmonary Bypass - Experimental and Clinical-Studies*. Annals of Thoracic Surgery, 1992. **54**(6): p. 1196-1202.

263. Whitaker, D.C., S.P. Newman, J. Stygall, C. Hope-Wynne, M.J.G. Harrison, and R.K. Walesby, *The effect of leucocyte-depleting arterial line filters on cerebral microemboli and neuropsychological outcome following coronary artery bypass surgery*. European Journal of Cardio-Thoracic Surgery, 2004. **25**(2): p. 267-273.

264. Stump, D.A., A.T. Rogers, J.W. Hammon, and S.P. Newman, *Cerebral emboli and cognitive outcome after cardiac surgery*. Journal of Cardiothoracic and Vascular Anesthesia, 1996. **10**(1): p. 113-119.

265. Moody, D.M., W.R. Brown, V.R. Challa, D.A. Stump, D.M. Reboussin, and C. Legault, *Brain Microemboli Associated with Cardiopulmonary Bypass - a Histologic and Magnetic-Resonance-Imaging Study*. Annals of Thoracic Surgery, 1995. **59**(5): p. 1304-1307.

266. Moody, D.M., M.A. Bell, V.R. Challa, W.E. Johnston, and D.S. Prough, *Brain Microemboli During Cardiac-Surgery or Aortography*. Annals of Neurology, 1990. **28**(4): p. 477-486.

267. Brown, W.R., D.M. Moody, V.R. Challa, and D.A. Stump, *Histologic studies of brain microemboli in humans and dogs after cardiopulmonary bypass.* Echocardiography-a Journal of Cardiovascular Ultrasound and Allied Techniques, 1996. **13**(5): p. 559-565.

268. Brooker, R.F., W.R. Brown, D.M. Moody, J.W. Hammon, D.M. Reboussin, D.D. Deal, H.S. Ghazi-Birry, and D.A. Stump, *Cardiotomy suction: A major source of brain lipid emboli during cardiopulmonary bypass.* Annals of Thoracic Surgery, 1998. **65**(6): p. 1651-1655.

269. Pugsley, W., L. Klinger, C. Paschalis, T. Treasure, M. Harrison, and S. Newman, *The Impact of Microemboli During Cardiopulmonary Bypass on Neuropsychological Functioning.* Stroke, 1994. **25**(7): p. 1393-1399.

270. Taggart, D.P. and S. Westaby, *Neurological and cognitive disorders after coronary artery bypass grafting.* Current Opinion in Cardiology, 2001. **16**(5): p. 271-276.

271. Mahanna, E.P., J.A. Blumenthal, W.D. White, N.D. Croughwell, C.P. Clancy, R. Smith, and M.F. Newman, *Defining neuropsychological dysfunction after coronary artery bypass grafting.* Annals of Thoracic Surgery, 1996. **61**(5): p. 1342-1347.

272. Hlatky, M.A., C. Bacon, D. Boothroyd, E. Mahanna, J.G. Reves, M.F. Newman, I. Johnstone, C. Winston, M.M. Brooks, A.D. Rosen, D.B. Mark, B. Pitt, W. Rogers, T. Ryan, R. Wiens, and J.A. Blumenthal, *Cognitive function 5 years after randomization to coronary angioplasty or coronary artery bypass graft surgery.* Circulation, 1997. **96**(9): p. 11-14.

273. Newman, M.F., J.L. Kirchner, B. Phillips-Bute, V. Gaver, H. Grocott, R.H. Jones, D.B. Mark, J.G. Reves, and J.A. Blumenthal, *Longitudinal assessment of neurocognitive function after coronary-artery bypass surgery.* New England Journal of Medicine, 2001. **344**(6): p. 395-402.

274. de Vries, A.J., Y.J. Gu, Y.L. Douglas, W.J. Post, H. Lip, and W. van Oeveren, *Clinical evaluation of a new fat removal filter during cardiac surgery.* European Journal of Cardio-Thoracic Surgery, 2004. **25**(2): p. 261-266.

275. Kincaid, E.H., T.J. Jones, D.A. Stump, W.R. Brown, D.M. Moody, D.D. Deal, and J.W. Hammon, *Processing scavenged blood with a cell saver reduces cerebral lipid microembolization.* Annals of Thoracic Surgery, 2000. **70**(4): p. 1296-1300.

276. Schmidt, H., J.O. Lund, and S.L. Nielsen, *Autotransfused shed mediastinal blood has normal erythrocyte survival.* Annals of Thoracic Surgery, 1996. **62**(1): p. 105-108.

277. Dalrymple-Hay, M.J.R., L. Pack, C.D. Deakin, S. Shephard, S.K. Ohri, M.P. Haw, S.A. Livesey, and J.L. Monro, *Autotransfusion of washed shed mediastinal fluid decreases the requirement for autologous blood transfusion following cardiac surgery: a prospective randomized trial.* European Journal of Cardio-Thoracic Surgery, 1999. **15**(6): p. 830-834.

278. Healy, W.L., B.A. Pfeifer, S.R. Kurtz, C. Johnson, W. Johnson, R. Johnston, D. Sanders, R. Karpman, G.N. Hallack, and C.R. Valeri, *Evaluation of Autologous Shed Blood for Autotransfusion after Orthopedic-Surgery.* Clinical Orthopaedics and Related Research, 1994(299): p. 53-59.

279. Dailey, J.F., *Blood.* 1998, Arlington: Medical Consulting Group.

280. Engstrom, K.G., *The embolic potential of liquid fat in pericardial suction blood, and its elimination.* Perfusion-Uk, 2003. **18**: p. 69-74.

281. Kaza, A.K., J.T. Cope, S.M. Fiser, S.M. Long, J.A. Kern, I.L. Kron, and C.G. Tribble, *Elimination of fat microemboli during cardiopulmonary bypass.* Annals of Thoracic Surgery, 2003. **75**(2): p. 555-559.

282. Klodell, C.T., J.D. Richardson, T.M. Bergamini, and D.A. Spain, *Does cell-saver blood administration and free hemoglobin load cause renal dysfunction?* American Surgeon, 2001. **67**(1): p. 44-47.

283. Nilsson, A., F. Petersson, and T. Laurell, *Improved design and performance of an acoustically operated multi channel particle separation chip* in *The International Conference on Miniaturized Systems for Chemistry and Life Sciences.* 2005. p. 575-577.

284. Toner, M. and D. Irimia, *Blood-on-a-chip.* Annual Review of Biomedical Engineering, 2005(7): p. 77-103.

285. Nilsson, A., F. Petersson, and T. Laurell, *Plasmapheresis in microfluidic channels using acoustic standing waves*, in *The International Congress on Ultrasonics*. 2007. p. 152-153.

286. Reents, W., J. Babin-Ebell, M.R. Misoph, A. Schwarzkopf, and O. Elert, *Influence of different autotransfusion devices on the quality of salvaged blood*. Annals of Thoracic Surgery, 1999. **68**(1): p. 58-62.

287. Murphy, G.J., S.M. Allen, J. Unsworth-White, C.T. Lewis, and M.J.R. Dalrymple-Hay, *Safety and efficacy of perioperative cell salvage and autotransfusion after coronary artery bypass grafting: A randomized trial*. Annals of Thoracic Surgery, 2004. **77**(5): p. 1553-1559.

288. Blevins, F.T., B. Shaw, C.R. Valeri, J. Kasser, and J. Hall, *Reinfusion of Shed Blood after Orthopedic Procedures in Children and Adolescents*. Journal of Bone and Joint Surgery-American Volume, 1993. **75A**(3): p. 363-371.

289. Vertrees, R.A., V.R. Conti, S.D. Lick, J.B. Zwischenberger, L.B. McDaniel, and G. Shulman, *Adverse effects of postoperative infusion of shed mediastinal blood*. Annals of Thoracic Surgery, 1996. **62**(3): p. 717-723.

290. Augustsson, P., F. Petersson, and T. Laurell, *Carrier medium exchange efficiency in acoustic standing wave particle washing*, in *The International Congress on Ultrasonics*. 2007. p. 154.

291. Grenvall, C., M. Carlsson, P. Augustsson, F. Petersson, and T. Laurell, *Fluorescence activated cell sorting using ultrasonic standing waves in microfluidic channels*, in *The International Conference on Miniaturized Systems for Chemistry and Life Sciences (submitted)*. 2007.

292. Kruger, J., K. Singh, A. O'Neill, C. Jackson, A. Morrison, and P. O'Brien, *Development of a microfluidic device for fluorescence activated cell sorting*. Journal of Micromechanics and Microengineering, 2002. **12**(4): p. 486-494.

293. Petersson, F., L.B. Åberg, and T. Laurell, *Simultaneous separation of multiple suspended particles by means of free flow acoustophoresis (FFA)*, in *The International Congress on Ultrasonics*. 2007. p. 150-151.

294. Vidal, S.D., J.P. Simonin, P. Turq, and O. Bernard, *Acoustophoresis revised*. Journal of Physical Chemistry, 1995. **99**(17): p. 6733-6738.

295. Heyman, J.S., *Acoustophoresis separation method*. 1992.

296. Laurell, T., J. Drott, and L. Rosengren, *Silicon-Wafer Integrated Enzyme Reactors*. Biosensors & Bioelectronics, 1995. **10**(3-4): p. 289-299.

297. Laurell, T., L. Wallman, and J. Nilsson, *Design and development of a silicon microfabricated flow-through dispenser for on-line picolitre sample handling*. Journal of Micromechanics and Microengineering, 1999. **9**(4): p. 369-376.