CONTROLLED MANIPULATION OF MICROPARTICLES UTILIZING MAGNETIC AND DIELECTROPHORETIC FORCES

LarsErik Johansson

2010

Mälardalen University Press Licentiate Theses
No. 123

School of Sustainable Development of Society and Technology
CONTROLLED MANIPULATION OF MICROPARTICLES
UTILIZING MAGNETIC AND DIELECTROPHORETIC FORCES

LarsErik Johansson

Akademisk uppsats

som för avläggande av teknologie licentiatexamen i bioteknik/kemiteknik
vid Akademin för hållbar samhälls- och teknikutveckling kommer att
offentligen försvaras måndagen den 15 november, 2010, 10.00 i
Selandersalen, Kv. Verktyget, Mälardalens högskola, Eskilstuna.

Opponent: Professor Leif Nyholm, Uppsala Universitet,
Ångström Laboratoriet, Institutionen för materialkemi.
Abstract

This thesis presents some experimental work in the area of manipulation of microparticles. Manipulation of both magnetic and non magnetic beads as well as microorganisms are addressed. The work on magnetic bead manipulation is focused on controlled transport and release, on a micrometer level, of proteins bound to the bead surface. Experimental results for protein transport and release using a method based on magnetization/demagnetization of micron-sized magnetic elements patterned on a modified chip-surface are presented. Special attention has been placed on minimizing bead-surface interactions since sticking problems have shown to be of major importance when protein-coated beads are used. The work with non-magnetic microparticles is focused on the dielectrophoretic manipulation of microorganisms. Preliminary experimental results for trapping and spatial separation of bacteria, yeast and non-magnetic beads are presented. The overall goal was to investigate the use of dielectrophoresis for the separation of sub-populations of bacteria differing in, for example, protein content. This was, however, not possible to demonstrate using our methods. Within the non-magnetic microparticle work, a method for determining the conductivity of bacteria in bulk was also developed. The method is based on the continuous lowering of medium conductivity of a bacterial suspension while monitoring the medium and suspension conductivities.
To my family

...closing the tweezers without moving the bacteria out of the way required patience...  
Jericho et al. 2004
List of papers

This thesis is based on the following papers:

I  “A magnetic microchip for controlled transport of attomole levels of proteins”

II “Determination of Conductivity of Bacteria by Using Cross-Flow Filtration”

III “A study of biological particles in Bio-Mems devices using dielectrophoresis”

IV “A Simple Open Micro System for Dielectrophoresis and Impedance Measurements”
   Mats Jönsson, Fredrik Aldaeus, Lars Erik Johansson, Ulf Lindberg, Johan Roeraade, Sven Hamp, Gunnar Jonsson
   *In manuscript.*
Author contribution

Paper I.
Major part of bead-transportation experiments and chemical modifications of bead- and chip surfaces. Significant part of planning, evaluation and writing.

Paper II.
Major part of experimental work. Significant part of planning, evaluation and writing.

Paper III
Major part of experimental work regarding dielectrophoresis. Part of planning, evaluation and writing.

Paper IV
Significant part of experimental work regarding dielectrophoresis. Part of planning, evaluation and writing.
The work has also been presented at the following conferences:

P1  “Transport of protein-covered magnetic beads at modified chip-surfaces”
Lars Erik Johansson, Klas Gunnarsson, Stojanka Bijelovic, Kristofer Eriksson, Emmanuelle Göthelid, Peter Svedlindh and Sven Oscarsson

P2  “Programmable Motion and Separation of Single Magnetic Particles on Patterned Magnetic Surfaces”
Lars Erik Johansson, Klas Gunnarsson, Erika Ledung, Sven Oscarsson, Peter Svedlindh.

P3  “Dielectric Characterisation of Microorganisms”
Sven Hamp, Lars Erik Johansson, Gunnar Jonsson, Fredrik Aldaeus, Mats Jönsson.

P4  “Escherichia coli behavior in an open dielectrophoretic microsystem”
Fredrik Aldaeus, Lars Erik Johansson, Mats Jönsson, Gunnar Jonsson, Ulf Lindberg, Johan Roeraade, Sven Hamp.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFM</td>
<td>Atomic Force Microscopy</td>
</tr>
<tr>
<td>DACS</td>
<td>Dielectrophoresis Activated Cell-Sorter</td>
</tr>
<tr>
<td>DEP</td>
<td>Dielectrophoresis</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribose Nucleic Acid</td>
</tr>
<tr>
<td>EDL</td>
<td>Electric Double Layer</td>
</tr>
<tr>
<td>FACS</td>
<td>Fluorescence Activated Cell-Sorter</td>
</tr>
<tr>
<td>HSA</td>
<td>Human Serum Albumine</td>
</tr>
<tr>
<td>MEMS</td>
<td>Micro Electromechanical System</td>
</tr>
<tr>
<td>MFM</td>
<td>Magnetic Force Microscopy</td>
</tr>
<tr>
<td>µTAS</td>
<td>Micro Total Analysis System</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>PEO</td>
<td>Polyethylene oxide</td>
</tr>
<tr>
<td>SPM</td>
<td>Scanning Probe Microscopy</td>
</tr>
<tr>
<td>STM</td>
<td>Scanning Tunneling Microscopy</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium Dodecyl Sulphate</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis</td>
</tr>
<tr>
<td>XPS</td>
<td>x-ray photoelectron spectroscopy</td>
</tr>
</tbody>
</table>
Introduction

The aim of this thesis is to present experimental results of some methods for the manipulation of microparticles. The term *particles* can stand for many things: for a particle physicist it can mean elementary particles whereas for a physical chemist it can mean nanoparticles which have dimensions below about 500 nm (the lower limit is not very well defined, but atoms and small molecules are not considered nanoparticles). Particles in the size range 0.5 – 100 µm are often referred to as *microparticles* and are, as the title indicates, an important component of this thesis. In the collective term microparticles, we can include cells, bacteria, large viruses, clay particles and dust to mention a few. Microparticles can be highly complex, such as a cell, or very simple, such as a silica particle, and a spectrum of complexities in between. Cells, bacteria and viruses are often referred to as bioparticles in the literature, especially in applied physics and microsystem technology.

In our everyday life, we often encounter microparticles, *e.g.* when brushing our teeth. Titanium dioxide (E171) microparticles make toothpaste look appealing due to its optical properties and are a part of the polishing process. Microparticles in foods are used for example as nutrient delivery vehicles to protect, for example, a sensitive vitamin in a food and release it in some part of the gastrointestinal system (Chen and Subirade, 2006). Another everyday example is glass microparticles that are used in road markers to improve light reflection (Swarco Vestglass GmbH). In pharmaceutical applications, microparticles can be used to control drug release by encapsulating the drug in the microparticle (microcapsules) or dispersing the drug through the matrix of the microparticle (microspheres) (Lasalle and Ferreira, 2007). The use of microparticles in bioanalysis is demonstrated in several papers, for example bar-coded particles, where each particle carries an individual readable code which enables immediate visual identification of that particles specific chemical functionalization (Pregibon, Toner and Doyle, 2007). In separation science, microparticles have been used for decades to build up chromatographic media (Porath and Flodin, 1959). By fine-tuning the size, matrix and surface chemistry of the particles it is possible to make chromatographic media for ion-exchange, reversed-phase, affinity, size-exclusion *etc.* Solid phase synthesis is another important area where molecules are synthesized on a microparticle surface. By having the synthesized molecule bound to the particle, reagents can easily be added and
by-products washed away with the product held in place. Today, chemical companies provide solid phase resins with a wide variety of surface functionalization. When the Norwegian researcher John Ugelstad developed monodispersed polymer microbeads and incorporated magnetic material in microbeads in the 1970s to 1980s, this led to a new means of separation in biotechnological applications. Since most biomaterial does not interact with magnetic fields, it became easy to selectively manipulate beads with a magnetic field without disturbing the rest of the material. Applications of magnetic beads include e.g. separation of whole cells, proteins and bacteria from complex samples (Vartdahl et al., 1986).

Manipulation of microparticles relative to their environment has been demonstrated by use of several techniques such as physical trapping, transportation, separation or rotation of the particle. Some methods for these types of manipulation are reviewed in this thesis, with some basic theory in a separate chapter. Due to their high surface/volume ratio, surface interactions can be a problem with micro- and nanoparticles, and thus a chapter on the basics of surface forces has also been included.

**Paper I** describes a novel system for the transport and release of proteins bound to magnetic beads performed on a chip-surface. **Paper II** describes a novel method for determining the conductivity of bacteria in bulk. **Paper III and IV** describe some preliminary studies of dielectrophoresis as a tool for manipulation of bacteria, yeast and beads.
1. Manipulation of micro-particles.

**Manipulation based on magnetism**

Magnetism-based methods for manipulation of (magnetic) beads range from mass-manipulation down to manipulation of single beads. For micro-scale manipulation of magnetic beads different strategies have been presented. A so-called unit operation of a magnetic bead-based micro-system is trapping of the magnetic beads. Smistrup et al. (2006) used micro-electromagnets placed below a flow-channel to capture, and subsequently release, a group of 1.1 µm diameter magnetic beads within the channel. Choi et al. (2000) also used micro-electromagnets to capture magnetic beads (1 µm diameter) from a fluid flow on a chip surface. Incorporated in their device was a sensing coil, which measured the inductance change as magnetic beads were trapped on the surface. A similar strategy was used by Ramadan et al. (2006ab) who used ferromagnetic pillars, magnetized by micro-electromagnets, to capture groups of magnetic beads (1-5 µm diameter) in a microfluidic device. The purpose of the pillars was to focus the magnetic field from the coils and locally create a high magnetic field gradient in order to enhance the trapping of beads. The magnetized pillar was situated outside of the flow channel containing the beads. Furthermore, a sensing coil was located close to the coil generating the trapping field. As a result of beads accumulating on top of the pillar, the inductance in the sensing coil changed and the presence of beads could thus be detected. Yellen and Friedman (2004) assembled patterns of magnetic beads in microwells on a chip by individually addressing magnetic elements with one end positioned within the well. A specific element can have its magnetization direction altered by selectively heating that element with a laser in the presence of an in-plane external magnetic field. By heating the element, it is can more easily have its magnetization direction altered by the external magnetic field than the non-heated elements, which are unaffected by the in-plane field. When the in-plane field is switched off, beads can be positioned in the well by binding to the element which have had its magnetization altered. The attraction of beads to the elements is due to a weak out-of-plane field which is always present in the experiment, and which vertical direction determines the attraction/repelling of beads to the elements. The “active” elements have their bead-attractive end positioned within the well. Once the
well is filled with beads, the element is switched off by changing back the magnetization direction. The beads remain within the well by ensuring to keep the vertical field weak enough to hinder the beads from being fully repelled. The presence of beads in the well also blocks it from contamination of other beads. Due to this, the chip can be rinsed and other elements can be switched on and other bead types can be bound to these.

Another type of unit operation of a magnetic bead-based micro-system is controlled movement of the magnetic beads relative to the environment. Lee, Purdon and Westervelt (2004) used microelectromagnets to demonstrate capture of single magnetic beads (2.1 µm diameter), yeast cells bound to a single magnetic bead (2.8 µm diameter) and magnetotactic bacteria (*Magnetospirillum magnetotacticum*). In the paper, the authors also demonstrated transportation of magnetic beads over a chip surface by the use of a matrix of microelectromagnets. In the latter experiment the beads were not controlled one-by-one, but as a small group. Deng et al. (2001) used a system of current-carrying micro-circuits to create localized magnetic field maxima on a micro-chip. By changing the location of the field maxima, groups of magnetic beads (4.5 µm diameter) were moved along the circuits. The field maxima were changed by altering the current direction in the circuits.

Janssen, van Ijzendoorn and Prins (2008) moved magnetic beads (1 – 2.8 µm diameter) between two current carrying wires on a chip. The beads were moved between the two wires by alternatively running a current through the two wires. The beads are magnetized by the field due to the wires and attracted by the field gradient. In the paper, the author also demonstrated a magnetic sensor, positioned in-between the two wires, which was capable of detecting the position of a single magnetic bead between the wires. Gunnarsson et al. (2005) used a line of soft magnetic elements in conjunction with a rotating magnetic field to transport single magnetic beads on a silicon surface. A bead follows the rim of an element synchronously with the rotation of the field until it reaches a position where it due to geometric reasons is more strongly attracted by a neighboring element and thus makes a jump to that element. In the same paper, a sorting mechanism is demonstrated, which enables a magnetic bead to shift into a side-track by reversing the rotation of the magnetic field. In this thesis, utilizing the method of Gunnarsson and co-workers, a novel method for controlled transport and release of proteins bound to magnetic beads is presented (paper I).
Manipulation based on dielectrophoresis

Dielectrophoresis was discovered in the 1950s by Herbert Pohl (Pohl, 1951) and is today used for micro- and nanoscale manipulation of various micro- and nanosized objects, of both synthetic and biological origin. A separate chapter describing some basics of dielectrophoresis is included in this thesis. Dielectrophoresis benefits from micro-fabrication technology, since quite high field strengths and gradients, two of the factors that determine the dielectrophoretic force, are achievable with low voltages when micro-electrode structures are used (Hughes, 2003). Low voltage demands (typically a few volts) provide a great advantage when building battery-driven, portable devices.

With dielectrophoresis it is possible to trap, or capture, objects ranging in size from a few nanometers to tenths of micrometer. It is also possible to use dielectrophoresis to create patterns of small objects on the surface or to move them around. Beck et al. (2008) used dielectrophoresis to trap single bacterial spores between two electrodes in order to perform electrical characterization of the spores. The authors were able to discriminate between spores of several Bacillus species by their current response at 10 kHz. The authors related the differences in electric response to differences in the surface chemistry of the spores, since some of the discriminated species were of a comparable size. Frénéa et al. (2003) used an electrode array to position mammalian cells in wells fabricated on a chip by placing the electrodes in a pattern so that the wells coincided with low-field regions where the cells experienced negative dielectrophoresis. Also demonstrated in that paper was the positioning of beads (3 µm diameter) in an electrode defined pattern. Alp, Stephens and Markx (2002) positioned bacteria and mammalian cells in a pattern on micro electrodes by utilizing positive dielectrophoresis. Layers consisting of up to 3 species vertically stacked or placed adjacent were constructed. Further, an artificial biofilm was constructed by polymerizing acrylamide on top of the structured species. Other examples are trapping of viruses (Morgan and Green, 1997; Green, Morgan and Milner, 1997) and sub-micrometer beads (Green and Morgan 1999). It is also possible to utilize dielectrophoresis to trap DNA and proteins at micro-electrodes (Washizu et al., 1994; Bakewell et al., 1998). Kawabata and Washizu (2001) demonstrated direct trapping of DNA and proteins at micro electrodes placed in a micro channel. Also demonstrated in the paper was the separation of an oligonucleotide (22 base-pairs) from
lambda-DNA (48500 base-pairs): The larger DNA-molecule is more readily attracted to the electrodes, since the dielectrophoretic force is size-dependent (see chapter 3). Other experiments in the paper use a DEP-enhancer, i.e. they selectively bind the target to a bead or molecule which can be manipulated by dielectrophoretic forces. Both the (obvious) use of a polystyrene bead and the use of lambda-DNA as the enhancer is demonstrated in the paper. Stretching of DNA between two electrodes has been demonstrated by Namasivayam et al. (2002). The authors connected one end of the DNA molecule to the edge of one of a pair of pointed micro-electrodes by chemical means. An AC-electric field was applied, thus making the DNA line up between the electrodes in a controlled way.

Dielectrophoresis can also be used for controlled movement of cells. Suehiro and Pethig (1998) manipulated plant protoplasts (30-50 µm diameter) in a controlled way in 2D by using a 3D grid of individually wired microelectrodes. In their system the cell is moved within the grid with the aid of locally induced negative and positive dielectrophoresis zones.

Dielectrophoresis-based separations have been studied for some decades and several types of separation have been demonstrated. In a paper by Markx and Pethig (1995), a system for continuous separation of cells is described. Live and dead yeast cells were separated based on differences in their dielectrophoretic response in combination with a fluid drag-force. The live cells were held tighter towards an electrode pattern than the dead cells and could therefore resist the fluid drag. A system of pumps and valves in combination with switching the field on and off made the system work in continuous mode. In another work by Markx, Dyda and Pethig (1996), different types of bacteria were separated based on their dielectrophoretic response. By varying the conductivity of the suspending medium they were able to selectively release one type of bacteria from an electrode pattern whereas the other type was still held in place. Recently, Pommer et al. (2008) demonstrated separation of platelets from diluted whole blood in a dielectrophoretic activated cell-sorter (DACS). The basis of the DACS described in the paper is the dielectrophoretic deflection of cells (larger than platelets) in a stream of the blood sample into a waste-stream.

Techniques related to dielectrophoresis are traveling wave dielectrophoresis and electrorotation. Traveling wave dielectrophoresis and electrorotation are, in turn, closely related to each other in the sense that both make use of a phase-shifted field to induce a torque on the particle to either transport it
along a lane of electrodes or rotate it on a spot, surrounded by the electrodes.
The books by Morgan and Green (2003) or Hughes (2003) are strongly recommended for the interested reader; however the theory of the subject is outside the scope of this thesis.
In this thesis, a novel method for determining the conductivity of bacteria in bulk is described (paper II). Also some preliminary work with dielectrophoretic manipulation of bacteria, yeast and beads are described (paper III and IV).

**Manipulation based on other forces**

**Optical tweezers** or **optical traps** exploit the fact that light exerts force on matter. Dielectric particles, such as uniform beads or bacterial cells, are attracted to and trapped near the waist of a laser beam that has been focused through a microscope objective. It is a so called *field gradient technique*, which also includes dielectrophoretic and magnetic tweezers.

When the particle is out of focus of the beam, it is exposed to a force pulling it back to focus (if the particle is transparent) or away from focus (if the particle is opaque). Furthermore, the particle will interact with the light-gradient in exactly the same way as in dielectrophoresis, causing it to move away from or towards high field gradients (Hughes, 2003). The forces acting on the particle are dependent on the refractive index, size and shape of the object as well as the intensity and wavelength of the beam. The refractive index is related to the dielectric constant (Nordling and Österman, 2004, p. 264).

Optical tweezers were invented during the 1970s by Arthur Ashkin (Ashkin, 1997) and have found great use in *e.g.* cell manipulation. The first reports on manipulation of particles in micron size were in 1986 by Ashkins group (Ashkin et al., 1986). In 1987 the same group reported trapping of microorganisms such as *Escherichia coli* and *Saccharomyces cerevisiae* (Ashkin, Dziedzic and Yamane, 1987). In *Lab-On-A-Chip* applications, the technique is gaining interest. Enger *et al.* (2004) used optical tweezers to move *E. coli* cells between different reservoirs in a micro chip. In the same publication, optical tweezers were used to trap a single *S. cerevisiae* cell in a flow of *E. coli* and *S. cerevisiae* and moved it into a side channel on the chip.

Optical tweezers have also been used in single molecule studies, *e.g.* stretching of DNA (Smith, Cui and Bustamente, 1996) and for studies of
protein properties on the molecular level (Tskhovrebova, Sleep and Simmons, 1997; Svoboda et al. 1993).

**Ultrasound** can be used to trap and position particles. Sound is basically a series of compressions and rarefactions of matter and its interaction with particles is depending on particle size, density and compressibility.

The group of Laurell used an ultrasonic standing wave (a wave that is “fixed” in its position in space) to separate red blood cells from lipids on a micro-chip (Nilsson et al., 2004; Petersson et al., 2005). The red blood cells were focused in the center of a micro-channel, where a pressure node was situated, whereas the lipids were pushed to the walls, where the pressure anti-nodes were situated.

Another work by the same group reports the capture of micro beads in a flow channel (Lilliehorn et al., 2005). Polystyrene beads (about 7 µm diameter) were captured in clusters on top of an ultrasonic transducer mounted in a flow channel.

**Mechanical** methods for manipulation are methods such as micromechanical tweezers, micro fluidic and SPM-techniques. Piezo-driven mechanical clamps mounted on a micro-manipulator were used by Jericho et al. (2004) to pick up and move *Staphylococcus aureus*, *Escherichia coli* and 1.1 µm latex beads on a glass slide. A commercial micro-manipulator with a stepping motor-controlled robotic arm onto which clamps, pipettes or probes can be mounted was used by Jericho’s group. On the molecular and nanometer scale, scanning probe microscopy (SPM) has been used to manipulate single molecules e.g. to stretch molecules (Rief et al., 1997; Marszalek et al. 1998) and to position nanoparticles (30 nm size) on a surface (Junno et al., 1995).

**Fluid mechanical** manipulating methods are often used in the fluorescence activated cell-sorter (FACS). The FACS is in principle a flow cytometer designed to identify (by fluorescence) and sort out specific cells from a fluid stream by controlling the flow direction. Efforts have been made to make the FACS an on-chip method for use in miniaturized diagnostic devices. Wolff et al. (2003) presented an on-chip FACS capable of sorting out fluorescent beads from a mixture of fluorescent beads and red blood cells. By opening a high-speed valve on the side-channel when a fluorescent bead passed a detector, the fluid flow (and the bead) could be directed into that channel. In the same paper, the authors also showed a prototype capable of sorting two populations of yeast cells, one containing green fluorescent protein (GFP)
and one normal. The population containing GFP were sorted to an on-chip cultivation chamber by the valve switching method described above.
2. Surface forces and Brownian motion.

**Surface forces**

In the systems investigated in this work a very intricate and interesting situation with forces acting between micro-particles (including beads, bacteria and cells) and the chip as well as between the particles themselves could be foreseen. The two main forces counteracting each other are the magnetic and electric forces which are the driving forces for transportation of particles and the “sticking forces” which will act against transportation. As long as the transportation of particles goes on, friction forces (particle-fluid and particle-surface) will also appear. All those forces involved in the techniques described in this thesis have not been experimentally determined since that is beyond the scope of this investigation. On the other hand, many references to other researchers work concerning different techniques for manipulation of micro-particles have been included and in most of these references surface forces have, at the most, been briefly addressed. Nevertheless, consideration of these forces is of crucial importance for this work, especially for the transport of proteins on beads, and thus a summary of most of the general existing knowledge of the forces involved at macromolecule/surface interfaces follows below (Israelachvili, 1985; Atkins, 2000; Norde, 2003).

**Intermolecular forces**

Intermolecular forces are effective at distances up to about 1 nm. As a comparison, a water molecule is about 0.14 nm in diameter, whereas a 30kD globular protein is about 7 nm in diameter. The forces discussed below are therefore considered short-range as they occur close to molecular contact.

**Ion-ion interaction**

The interaction energy, $w$, between two charges, $Q_1$ and $Q_2$, a distance $r$ apart is given by Coulomb's law,

$$w(r) = \frac{Q_1 \cdot Q_2}{4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon \cdot r}$$
where $\varepsilon$ is the dielectric constant of the medium between the charges. It follows from the equation that the interaction energy decreases with the inverse of the distance between the charges and with increased dielectric constant of the medium between the charges. Polar solvent molecules such as water ($\varepsilon = 80$) tend to orient around ions, which causes a decrease of the ion-ion interaction energy since some of the available energy is needed to keep the solvent molecules oriented. In addition, ions are also surrounded by nearby oppositely charged ions which screen the charge. These effects make the ion-ion interaction quite short range.

**Hydrogen bonding**

If a hydrogen atom is bond to an electronegative atom, it tends to be polarized and possess a partial positive charge. The partially charged hydrogen can interact with other electronegative atoms situated on nearby molecules or within the same molecule. The hydrogen bond between two molecular groups, $E_1$–$H$ and $E_2$, is generally described as

$$E_1\text{--}H\text{--}\text{- - -}E_2$$

where $E_1$ and $E_2$ are two electronegative atoms and the hydrogen bond is present between the hydrogen (which is covalently bond to $E_1$) and $E_2$. Examples of molecules capable of forming hydrogen bonds are water, DNA and proteins. In bulk water, each water molecule can interact with four other water molecules and thus form a three-dimensional network. The basic building block in the network is an oxygen atom surrounded by four hydrogen atoms, two covalently bond to the oxygen, two bound via hydrogen bonding. There is a constant reorganization of hydrogen bonds in aqueous solutions.

**Hydrophobic interaction**

If a molecule incapable of forming hydrogen bonds is introduced in water, the water molecules will structure themselves around the molecule. This structuring lowers the entropy of the water molecules, and is thus thermodynamically unfavorable. If more of these molecules are introduced, they tend to aggregate to minimize the exposed surface towards water and thus minimizing the amount of structured water. This entropy-based
interaction between molecules in water is known as the hydrophobic interaction.

**Ion-dipole interaction**

A dipole consists of two opposite charges separated by a distance. If placed close together with a charge \( (Q) \), a fixed dipole will interact by both a repulsive and an attractive force since the dipole possesses two different charges. The geometrical position of the dipole relative to the charge as well as the dipole moment \((u)\) will influence the interaction energy, \( w \), as described by the (approximate) expression

\[
w(r, \theta) = - \frac{Q \cdot u}{4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon} \cdot \frac{\cos(\theta)}{r^2}
\]

where \( \cos(\theta) \) is the angle between the field line from the charge and the direction of the dipole. The direction of the dipole is (by definition) from the negative to the positive end, whereas the electric field line is directed (by definition) from positive to negative potential. From this follows the minus sign (attractive force) at angles between 0-90° and 270-360°. Attractive force means that the dipole has its oppositely charged end closest to the charge of interest. The above expression is valid when the dipole is small compared to the distance from the charge.

If the dipole can rotate freely, the interaction energy, \( w \), will also be dependent on thermal energy according to

\[
w(r) = - \left( \frac{Q \cdot u}{4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon} \right)^2 \cdot \frac{1}{6 \cdot k \cdot T \cdot r^4}
\]

with the notation as above, \( k \) being the Boltzmann constant and \( T \) being the absolute temperature. As in the previous expression, the dipole must be small compared to the distance from the charge for the expression to be valid.
Dipole-dipole interaction
If two nearby dipoles, $u_1$ and $u_2$, are fixed at an arbitrary orientation in space, the interaction energy, $w$, between the two dipoles will be given by

$$w(r) = -\frac{u_1 \cdot u_2}{4 \pi \varepsilon_0 \varepsilon} \cdot \frac{\text{const}}{r^3}$$

where the constant, $\text{const}$, is dependent on the relative orientation of the dipoles in space: Consider one of the dipoles restricted to rotation ($\theta_1$) around its center in a plane, then place the other dipole with its center fixed in the same plane, at the distance $r$, and allow that dipole to rotate both in the plane ($\theta_2$) and out of the plane ($\phi$). This arrangement will allow all possible orientations between the two dipoles and the constant then becomes

$$\text{const}(\theta_1, \theta_2, \phi) = 2 \cdot \cos(\theta_1) \cdot \cos(\theta_2) - \sin(\theta_1) \cdot \sin(\theta_2) \cdot \cos(\phi)$$

which can take values between 2 (in line orientation, same direction) and -2 (in-line orientation, opposite direction).

Induced dipole interactions
As seen above, permanent dipoles are affected by electric fields, e.g. the electric field from an ion causes nearby dipoles to orient parallel to the field. Further, if any atom or molecule is placed in an electric field, its surrounding electrons will be affected by the field in such a way that the electron cloud is displaced relative to the center of positive charge, thus producing an induced dipole. The orientation of a permanent dipole as well as the build-up of an induced dipole in an electric field both fall under the concept polarization. This concept is also important in the theory of dielectrophoresis, discussed in a separate chapter.

The polarizability, $\alpha$, of an atom or a molecule is a measure of its tendency to become polarized in an electric field, $E$, given by the expression

$$\alpha = \frac{u}{E}$$
where \( u \) is the induced dipole moment in the molecule or atom. The dipole can be induced by the field from an ion or a dipole (permanent or induced). The charge \( Q \)-induced dipole \( \alpha \) interaction is described by

\[
w(r) = -\left( \frac{Q}{4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon} \right)^2 \cdot \frac{\alpha}{2 \cdot r^4}
\]

The dipole-induced dipole and induced dipole-induced dipole interactions are described in the following section.

**Van der Waals interaction**

The van der Waals interaction is in fact three types of interactions involving permanent and induced dipoles with the common property of \( 1/ r^6 \) decay in interaction energy.

The **Keesom interaction** is the interaction between two freely rotating permanent dipoles, \( u_1 \) and \( u_2 \), described by the expression

\[
w(r) = -\left( \frac{u_1 \cdot u_2}{4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon} \right)^2 \cdot \frac{1}{3 \cdot k \cdot T \cdot r^6}
\]

The **Debye interaction** is the interaction between two freely rotating permanent dipoles, \( u_1 \) and \( u_2 \) with polarizabilities \( \alpha_1 \) and \( \alpha_2 \).

\[
w(r) = -\frac{u_1^2 \cdot \alpha_2 + u_2^2 \cdot \alpha_1}{(4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon)^2} \cdot \frac{1}{r^6}
\]

The **London dispersion interaction** is an interaction always present between any two molecules or atoms due to temporal dipoles induced by vibrational displacement of electrons in the molecules or atoms. The interaction is of quantum mechanical nature and a full description is beyond the scope of this brief summary, but a reasonable approximation for the interaction between two atoms/molecules is the expression

\[
w(r) = -\frac{\alpha_1 \cdot \alpha_2}{(4 \cdot \pi \cdot \varepsilon_0 \cdot \varepsilon)^2} \cdot \frac{I_1 \cdot I_2}{I_1 + I_2} \cdot \frac{3}{2 \cdot r^6}
\]
where $\alpha_x$ and $I_x$ are the polarizability and first ionization potential of each atom/ molecule. As with the other interactions described above, the medium between the interacting molecules affects the interaction energy.

**Born repulsion**

As two atoms/molecules approach, there will be a distance close to contact where the molecular orbits start to overlap. This is, however, not allowed since the Pauli exclusion principle forbids two electrons to be within same orbital with the same spin and therefore there will be a strong repulsion closer than this distance. The repulsion follows the intermolecular distance, $r$, approximately as $1/r^{12}$, whereas the dispersion attraction follows $-1/r^6$ as discussed above. This is summarized in the Lennard-Jones potential

$$w(r) = \frac{A}{r^{12}} - \frac{B}{r^6}$$

where $A$ and $B$ are constants. This means that there exists a certain distance where the interaction energy is minimal. The Born repulsion implies that atoms does not simply collapse into each other by dispersive attraction, *i.e.* the attractive and repulsive forces balances each other.

The molecular interactions discussed above are so called pair-potentials, *i.e.* it is the interaction between two molecules which are described. In the case of a molecule approaching a surface, it is under the influence of numerous other molecules at the surface. Moreover, in the case of two approaching surfaces the number of possible interactions are further increased. In the following section the effect of these types of mass-interactions are summarized.

**Colloids**

A colloid is a phase that is heterogeneously dispersed in a continuous phase, for example polymer beads in water or fat-particles in milk. Colloids are typically in the size-range of a few nm to a few µm and thus have large surface/volume ratios which, in turn, make interfacial phenomena of great importance. In the experiments in paper I-IV, particles in the size-range 1-5
μm are used, and thus the surface effects must be taken into account for these systems.

Electric double-layer (EDL) interaction
A surface can acquire a charge by *e.g.* ionization of groups such as amines and carboxylic acids present on the surface. When a charged surface is immersed in an electrolyte solution, oppositely charged ions become attracted to the surface. This is energetically favorable, since the charge on the surface is screened but at the same time entropically unfavorable because of the build-up of an excess of positive or negative ions close to the surface. The closer the approach to the surface, the more important the charge-screening effect becomes whereas farther from the surface the entropic effect is more important. The consequence of this duality is that the ion-distribution will change as we approach the surface from the bulk. The electric double layer is the layer where the ion-distribution changes from that in the bulk (Figure 1).
Figure 1. Sketch of the electric double layer at a negatively charged surface immersed in an electrolyte solution. The ion distribution in the double layer is asymmetric, with a larger fraction of positive ions close to the negatively charged surface than in the bulk. The electric potential changes from the surface to the bulk according to the curve in the figure (Note: inverted ordinate [positive direction downwards]).

The curve in Figure 1 describes the electric potential, $\psi$, at different distances ($x$) from the surface according to

$$\psi (x) = \psi_0 \cdot e^{-\kappa \cdot x}$$

where $\psi_0$ is the surface potential. The factor $1/\kappa$ is the Debye length, which is (by definition) the thickness of the diffuse double layer. The thickness of the EDL is dependent on the ion concentration. Higher concentration of ions in the bulk makes the double layer extending a shorter distance out of the surface (more effective charge screening), see further below (Figure 2). The above EDL model was developed by Gouy and Chapman and serves well as
a simple and intuitive model of the double layer. The model was refined by Stern, who assigned the innermost layer of ions a different property, in which the potential drops linearly.

The effect of the EDL on interactions between surfaces differs depending on the charge of the surfaces. Two like surfaces will repel each other when the double layers starts to overlap because of the increased ion concentration in between the surfaces. Two oppositely charged surfaces will attract, since the charges on the surfaces can interact and therefore the ions in the EDL can be released (and thus increasing the entropy of the system). A charged and an uncharged surface will repel each other, since the uncharged surface will hinder the build-up of the EDL when closer than the Debye length.

**Combined electric double layer and van der Waals interaction**

In the 1940s, Derjaguin, Landau, Verwey and Overbeek described the stability of lyophobic\(^1\) colloids in suspension in terms of electric double-layer and dispersion forces. This is summarized in the DLVO-theory where the total interaction energy between two colloids is the sum of the dispersion interaction energy and the electric double layer interaction energy. If two similar lyophobic surfaces are brought together in an electrolyte solution the electric double layers will act repulsively due to the increased ion concentration in between the surfaces as they approach each other, whereas the dispersion forces will act attractively. This can be demonstrated with interaction curves as shown in Figure 2.

---

\(^1\) The term lyophobic means that the dispersed particle does not interact with ("like") the surrounding medium in which it is dispersed.
Figure 2. DLVO interaction between two similar surfaces approaching in electrolyte solutions of increasing ionic strength (left → right). The upper (blue, dashed) curve is the EDL-interaction (repulsive), the lower (red, dashed) curve is the dispersion interaction (attractive), the middle (solid, black) curve is the total interaction. The abscissa represents the distance between the two surfaces.

In Figure 2, the repulsive double layer interaction decreases with increasing ionic strength (left → right), due to the shielding effect of the ions on the surface charge. The attractive dispersion interaction remains constant and the total interaction is therefore attractive at all distances at high enough ionic strength (Figure 2, right). At close contact the Born-repulsion will cause a large repulsion of the surfaces, not shown in the figure (see discussion above).

Steric effects and deviation from DLVO-theory
Refinements of the DLVO-theory take into account solvation and steric effects, which can manifest themselves as deviations from DLVO-behavior, e.g. oscillations in the force as the surfaces approach or repulsions/attractions not expected from pure double-layer or dispersion interactions. One such example is the influence of polymers present on the surfaces. If the polymers are readily soluble in the media in between the surfaces there will be a repulsive force, due to decreased entropy and increased osmotic pressure\(^2\), which follows when the polymers are compressed as the surfaces approach (Figure 3). Increased attraction between the polymer surfaces can also occur if the solubility of the polymer

---

\(^2\) Osmotic pressure is the pressure which makes water flow from diluted regions to regions with higher amount of solutes.
is low or the surface coverage of the polymer is poor. This can be due to entangling of the polymers, if they have a greater affinity to each other than for the solvent or due to adsorption of polymer molecules on both surfaces if the surface coverage is poor.

Figure 3. Steric repulsion of two surfaces coated with polymers readily soluble in the media in between the surfaces. The dashed blue curve is the EDL-interaction (repulsive), the dotted blue curve is the steric interaction due to the presence of polymers at the surfaces (repulsive), the dashed red curve is the dispersion interaction (attractive), the solid black curve is the total interaction (attractive → repulsive). The abscissa represents the distance between the two surfaces.

In the protein transport experiments, described in paper I, the surface of the chip as well as the beads have been coated with polyethylene oxide (PEO) to reduce interaction between the beads and the chip as well as to avoid conformational changes of the proteins bound to the beads (Norde, 2003).
The good solubility of PEO in water promotes steric repulsion of the chip and bead surfaces due to non-DLVO repulsion.

**Brownian motion**

As the size of an object goes from centimeter or millimeter size down to the size of molecules, it will also change its physical behavior. The effect of random walk of a grain of sand is almost non-existent, whereas that of a hydrogen molecule is very large. It is somewhere in between these limits that micro- and nanotechnology has its domain. The randomizing effect of Brownian motion counteracts the electric-, magnetic- and surface-forces discussed above. The work described in this thesis is mostly concerned with micrometer-sized beads, so in the following section the random movement of a representative bead is described.

Brownian motion is the random motion of small particles that follows as a result of their collision with surrounding molecules. The mean displacement $x$ of a particle due to Brownian motion is described by the equation

$$x = \sqrt{2D \cdot t}$$

where $t$ is the available time and $D$ is the diffusion constant, which is dependent on temperature as well as the particle shape. For a spherical particle, the diffusion constant is calculated by the equation

$$D = \frac{k \cdot T}{6 \cdot \pi \cdot \mu \cdot a}$$

where $k$ is the Boltzmann constant, $T$ is the absolute temperature, $\mu$ is the dynamic viscosity and $a$ is the particle radius.

The mean displacement in 1 s due to Brownian motion for a particle with radius 5 µm in water at 298 K is about 0.3 µm (or 6% of the particle diameter). As the particle size is reduced, the displacement increases. For a 1 µm particle the above calculation gives a mean displacement of 0.7 µm (or 70% of the particle diameter). The dynamic viscosity for water at 298 K is about $10^{-3}$ Ns/m². The Boltzmann constant is of the order $1.38 \cdot 10^{-23}$ J/K.

In paper I, III and IV particles between 1 and 5 µm are used. Some effects of Brownian motion can therefore be expected in those experiments.
3. Dielectrophoretic and magnetic forces

Dielectrophoresis

Dielectrophoresis is the phenomenon of motion of polarizable particles in non-uniform electric fields discovered by Herbert Pohl in 1951 (Pohl, 1951). Dielectrophoresis differs from electrophoresis in some fundamental aspects. Electrophoretic motion is induced by the Coulomb force acting on any charged object in a homogenous or non-homogenous electric field, whereas dielectrophoretic motion is induced by the force between dipoles in the object and a non-homogenous electric field. The most important differences are that for electrophoresis to work, the object needs to be charged, whereas for dielectrophoresis, the object must be polarizable (it can be either charged or uncharged). Finally, electrophoresis is performed in DC fields, whereas dielectrophoresis can be performed in both AC and DC fields.

Dipoles

Dipoles can be of two types: permanent or induced. In a permanent dipole, the dipole exists both in the presence and the absence of an electric field and is dependent on the atomic configuration of the molecule. Water and carbon monoxide are examples of molecules possessing permanent dipoles. Dipoles can also be induced in molecules: the electron cloud surrounding a molecule can be displaced relative to the nuclei (electronic polarization), or a charge can move between different positions in the molecule (atomic polarization), thus creating a dipole. In an induced dipole, the dipole can exist only in the presence of an electric field. By convention, dipoles are sketched from negative to positive ends.

Polarization

Polarization is a process in which a dipole aligns in an electrical field. In the case of a induced dipole, the polarization occurs as the dipole is induced (see above). A molecule possessing a permanent dipole can also align in the field by rotation of the molecule (orientational polarization). These polarization mechanisms are referred to as Debye polarizations. At higher frequencies of the alternating field, orientational polarization is not induced. For example, in the case of water, frequencies greater than a few GHz can not induce orientational polarization due to inertia of the water molecules.
Consequently, the water molecule cannot reorient itself synchronously with the field. This causes a so called dispersion, which means that the orientational contribution to the total polarization is lost and only those polarization mechanisms with shorter relaxation times (atomic and electronic) contribute. Thus, as a polarization mechanism is lost in a material, the permittivity (dielectric constant) of the material is lowered.

In dielectrophoretic applications the frequencies are in the lower range, kHz to MHz, and the above-mentioned polarization mechanisms (Debye, electronic and atomic) can be considered constant. Instead another polarization mechanism, the Maxwell-Wagner interfacial polarization, dominates. This type of polarization occurs when two materials with different polarizability share an interface (such as a particle suspended in a medium) are exposed to an alternating electric field. As a result of the two materials polarizing or conducting charge differently, there will be a charge build-up, polarization, in the interface between the two materials. In Figure 4, this process is shown for three cases: a particle with less, equal and higher polarizability than the surrounding medium placed in an electric field. In the case of an particle which polarize more easily than the surrounding medium, more charges are built-up on the inside than on the outside of the particle-surrounding interface (Figure 4, right). In the case of an particle which polarize less easily than the surrounding medium, more charges are built-up on the outside than on the inside of the particle-surrounding interface (Figure 4, left). The net induced dipole thus changes direction depending on the relative polarizabilities of the particle and the surrounding medium. If the electric field is reversed, the charges will move to the opposite sides, thus the induced dipole also reverse. At high enough frequencies the movement of free charges are too slow to keep pace with the change in electric field direction, and a relaxation thus occur. The remaining polarization mechanisms are then those with higher relaxation frequencies. These responses of the particles to the electric field are the basis of dielectrophoresis, as will be discussed in the following section.
The dielectrophoretic force

When a dipole is induced in a particle due to an external electric field according to the polarization mechanisms discussed above, there are two possibilities of what may happen to the particle from a dielectrophoretic point of view:

1. If the particle is placed in a homogenous field the net force on the dipole will be equal at both poles, and the particle will stay in place, i.e. nothing happens.
2. If the particle is placed in a non-homogenous field the force on the dipole will be unequal at the poles, and the particle will move. The direction of the movement will be determined by the direction of the induced dipole.
If the dipole is aligned with the field (Figure 4, right), the dipole is attracted towards higher field-strengths. If the dipole is opposing the field (Figure 4, left), the dipole is attracted towards lower field-strengths.

The dielectrophoretic force-vector, \( F_{\text{DEP}} \), on a dipole is given by

\[
F_{\text{DEP}} = (p \cdot \nabla)E
\]

where \( p \) is the induced dipole moment, \( E \) is the electric field and \( \nabla \) is the del operator\(^3\). If the field is changing in space (\( i.e. \) the gradient is non-zero), there will be a net force on the dipole.

If applied to a particle, the dielectrophoretic force vector will depend on

1. the electric field properties such as, strength, curvature and frequency,
2. the particle properties such as size, shape and dielectric properties,
3. the dielectric properties of the surrounding medium.

The general expression for the dielectrophoretic force on a solid spherical particle suspended in a medium is (Morgan and Green, 2003; Hughes, 2003)

\[
F = 2\pi \varepsilon_m r^3 \cdot \text{Re}[K(\omega)] \cdot \nabla |E|^2
\]

where \( \varepsilon_m \) is the relative permittivity (dielectric constant) of the surrounding medium, \( r \) is the radius of the particle, \( E \) is the electric field (V/m) and \( \text{Re}[K(\omega)] \) refers to the real part of the complex Clausius-Mossotti factor (Morgan and Green, 2003; Hughes, 2003),

\[
K(\omega) = (\varepsilon_p^* - \varepsilon_m^*)/(\varepsilon_p^* + 2\varepsilon_m^*)
\]

where \( \varepsilon_p^* \) and \( \varepsilon_m^* \) refer to the complex permittivity of the particle and medium respectively. The complex permittivity is the permittivity corrected for the frequency dependence present in so-called lossy dielectrics. This

---

3 The del-operator, \( \nabla \), is the multi-dimensional analogy to the derivative. In three dimensions \( \nabla \) is defined as \( (\partial/\partial x, \partial/\partial y, \partial/\partial z) \). When applied to a vector field, the del-operator gives the direction and magnitude of the greatest slope of the field (gradient).
means that the dielectric contains both a capacitive and a resistive component. In electrical terms this is in principle a capacitor and a resistor in parallel. At low frequencies the impedance is determined by the resistive component, whereas at high frequencies the impedance is determined by the capacitive component (see discussion of polarization above). The real part of the Clausius-Mossotti factor can take numbers between -0.5 and 1, which means that this factor is of importance in determining the direction of the dielectrophoretic force (Figure 5).

Figure 5. The real part of the Clausius-Mossotti (CM) factor at different applied frequencies. The sign of the CM factor changes from positive to negative slightly above $10^6$ Hz, which means that the particle shows negative dielectrophoresis above this frequency.

If the real part of the CM-factor is positive, the force-direction is towards high field strengths, whereas if it is negative it is directed towards low field strengths. In Figure 4, this is represented by the change in dipole direction from parallel to anti-parallel at increased frequencies (the particle behaves as an capacitor at high frequencies since the free charge-movement is too slow to keep pace with the field). This frequency dependence is of great importance, since it makes it possible to separate objects with different dielectric properties by choosing a frequency where Re[K(ω)] have different
signs for the two objects (Gascoyne and Vykoukal, 2002; Morgan and Green, 2003; Hughes, 2003).

**Positive dielectrophoretic trapping**
Once trapped by positive dielectrophoresis, a particle is moved towards higher field strength, and thus the trapping becomes stronger with time (until it is stopped *e.g.* by reaching the electrode edge) as the force on the particle is increased with the field strength (Hughes, 2003).
Magnetism

Magnetic field
Magnetic fields are generated by moving electric charges. One example is the magnetic field around a current carrying wire (Figure 6), where the magnetic field is produced by a net flow of electrons in the wire. Another example is the magnetic field around a bar magnet (Figure 7). Here, the origin of the magnetic field is the orbital and spin moment of the electron.

Figure 6. Magnetic field produced by an electric current in a wire.

Figure 7. Magnetic field produced by a bar magnet.

Magnetic field intensity, magnetic flux density, magnetization and magnetic susceptibility.
The magnetic field generated by the current in a wire as in Figure 6 is called the magnetic field intensity or simply the magnetic field, \(H\) (A/m). When the magnetic field is applied to a particular medium, the responding field is called the magnetic flux density, \(B\) (Vs/m\(^2\); Tesla, T). The relation between \(B\) and \(H\) is expressed as

\[
B = \mu_0(H+M),
\]

where \(\mu_0 = 4\pi \cdot 10^{-7}\) (N/A\(^2\)) is the permeability of free space and \(M\) (A/m) is the magnetization of the material defined as

\[
M = \chi H
\]

\(\chi\) (-) is called the magnetic susceptibility of the material.

Combining the two above equations yields

\[
B = (X+1)H
\]

or

\[
B = \mu_r\mu_0H,
\]

where the relative permeability, \(\mu_r\), (-), is related to \(X\) by

\[
\mu_r = (X+1).
\]

For a non magnetic material, e.g. in air, \(X = 0\) (or \(\mu_r = 1\)) and \(B\) is proportional to \(H\).

The origin of magnetization in a material is of quantum mechanic origin and a full description of the phenomenon is outside the scope of this thesis, but a simplified model is given below.

**Magnetic dipoles and magnetization**

The electric dipole is, as described earlier, defined as two electric charges, electric monopoles, separated by a distance. The strength of the electric
dipole can then be defined as the product between the separated charge and the distance and has the unit [C*m].

Since magnetic monopoles are not defined, the description of a magnetic dipole is somewhat different from that of an electric dipole:
A magnetic dipole can be defined by a circular loop carrying an electric current, thus producing a magnetic field normal to the loop plane. The strength of the magnetic dipole can then be defined as the electric current through the loop times the area inside the loop and has the unit [A*m²].

In the classical atomic model, the electron orbiting the nucleus contributes to the total magnetic moment of the atom (or ion) in two separate ways:

- The orbital magnetic moment which is the effect of the electron orbiting the nucleus.
- The electron spin magnetic moment which is the effect of the electron spinning around its own axis.

For the transition elements, e.g. iron, the electron spin magnetic moment gives the major contribution to the total magnetic moment. In magnetic elements, the moments of the electrons add up to a total non-zero moment for the atom (or the ion). Hence, each atom (or ion) acts as a magnetic moment, or dipole. The magnetization is the total magnetic moment per volume in a material, thus it is given in [A/m].

Ferro-/ferrimagnetic materials and magnetic domains.
The dipolar interaction tends to order magnetic moments anti-parallel (cf. the behavior of two macroscopic bar magnets). However, in a ferromagnetic material, the neighboring atomic moments are ordered in parallel, due to a quantum mechanical based, short range interaction, called the exchange interaction.

This long-range/short-range interaction duality causes an energy trade-off which manifests itself in the following way:

In the ferromagnetic material there are areas, called magnetic domains, in which the magnetic moments are ordered in parallel due to the short range interaction. This causes a net magnetization of the material within the domain. However, due to the long-range dipolar interaction, striving towards the anti-parallel configuration, neighboring domains are ordered in other directions, which causes a zero total moment of the material (in the demagnetized state).
When the material is exposed to an external magnetic field, the domains with magnetization directions close to the applied field will increase in size (a large enough field can also change the magnetization direction within the domain). When the field is removed, the enlarged domains will not completely return to their original size. This is called magnetic remanence, and causes the material to keep a total bulk magnetization, even in the absence of the field.

The ferrimagnetic materials differ from ferromagnetic materials by the short-range ordering of magnetic moments, which are divided in both parallel and anti-parallel configuration in the ferrimagnetic material. There is, however, an imbalance in this ordering in the ferrimagnetic materials which causes a magnetic moment.

The introduction of magnetic domains in a material will also introduce areas, domain walls, within the material where the magnetization direction changes. The introduction of a domain wall costs energy since a material is more easily magnetized along certain directions, easy axes, determined by its crystal structure. If the atomic moments are forced to be directed in non-easy directions, as in a domain wall, the so-called magnetocrystalline anisotropy energy increases with the number of atomic magnetic moments. This will tend to make the wall thin. If the change in moment direction between adjacent atoms is small, there is less cost in exchange energy between adjacent atoms. This will tend to make the domain wall thick, since more atoms are needed in the domain wall to make the change in direction between each atom small. Therefore, the domain wall thickness will be a bargain between minimized anisotropy energy and minimized exchange energy. If a magnetic crystal is small enough, it cannot contain a domain wall since the wall must be of a finite thickness. The result of this is a single domain crystal, i.e. all magnetic moments point in the same direction. The size of a single domain is material-dependent, but about 50 nm is common.

Superparamagnetism
When the size of a single domain is reduced, there will be a point where it cannot keep the magnetization direction when the magnetizing field is removed and the magnetization will constantly change direction and behave very much like a large paramagnetic atom. When the single domain particle

---

4 Paramagnetic atoms have unpaired electrons, which cause a net magnetic moment in the atoms.
shows this behavior it is said to be superparamagnetic. The size of a superparamagnetic particle is material-dependent, but sizes below about 15 nm are common. Superparamagnetic particles are important in magnetic bead technology, because of their lack of magnetic remanence.
4. Methods

Electric conductivity measurement

When an electric potential \((U)\) is applied over a piece of conducting material, there will be a drift of charges in the field due to the electric potential. This drift of charges, the electric current \((I)\), is related to the potential by Ohms law

\[
I = K \cdot U
\]

where the conductance, \(K\), describes how much charge a specific piece of material transports at a given potential. Its reciprocal is the parameter resistance \((R)\), which give Ohms law the familiar form

\[
U = I \cdot R
\]

The resistance can be expressed as

\[
R = \rho \cdot \frac{l}{A}
\]

where \(l\) is the length of the sample, \(A\) is the cross-section and \(\rho\) is the resistivity, which is a material-specific parameter. In terms of conductance the expression takes the form

\[
K = \kappa \cdot \frac{A}{l}
\]

where \(\kappa=1/\rho\) is the conductivity. Electric charge can be transported as free electrons, as in metals, or as mobile ions, as is in an electrolyte solution. A conductivity meter for measurements on electrolyte solutions consists in its simplest case of a measurement cell, an electric power source and some type of resistance analyser (Figure 8).
Figure 8. Principle of a conductivity meter. The resistance (or conductance) of the solution is measured with a voltage and current meter (by Ohms law). With knowledge of the cell constant, given by the geometry of the cell, the conductivity can be calculated. Figure courtesy of Endress+Hauser, Germany.

The geometric term, $A/l$, is called the cell constant, and depends on the sensor geometry. If the cell constant is known, the conductivity can be determined by a simple current-voltage measurement. The cell constant can be determined experimentally by measurements on conductivity standards.

In paper II, a conductivity meter (Conducta CLS-TSP 3567, Endress+Hauser, Germany) has been used to measure the conductivity of bacterial suspensions.

To avoid redox reactions at the electrodes of the conductivity meter, an alternating current with a frequency in the kHz range is used for the measurements. The value obtained will be the impedance with frequency-dependent contributions from capacitive and conductive elements in the solution. The measuring frequency is set by the software in the instrument. Since the conductivity is temperature-dependent, about 2 %/°C, the temperature must be under strict control. During the measurements, the temperature is controlled by both a built-in temperature probe in the conductivity sensor and by an external PT-100 sensor. Prior to the
measurements, the conductivity meter is calibrated with known standards, spanning the conductivity range.

**Cross-flow filtration**

Filtration is a solid-fluid separation process where materials are separated depending on their ability to pass through a filter. Filtration is used for separation of materials ranging in size from several micrometers, such as particulate material, yeast and bacteria in micro-filtration processes, via large molecules *e.g.* proteins in ultra-filtration processes down to simple sugars and small ions in nano-filtration and reverse osmosis processes (Cheryan, 1998).

Depth-filtration is a well known filtration method, where the fluid-flow is normal to the filter. The syringe filter, found in most laboratories, is an example of a depth filtration unit. This type of filter set-up has the advantage of being simple to fabricate and use, but has a draw-back in the build-up of a filter-cake which decreases filtrate flow as it grows in thickness. In industrial applications, systems for back-flush and mechanical methods are used to remove the filter-cake.

Cross-flow filtration is a filtration process where the material to be filtered is continuously circulated tangentially over a filter membrane. Figure 9 is a schematic of a cross-flow filtration system. The material to be filtered is contained in the process tank (A) and pumped (C) over a filter membrane contained in the filter module (D). The retentate is taken back to the process tank via the retentate stream (F) and the filtrate is taken out from the filter module via the filtrate stream (E). The diafiltrate stream (G) is used for washing and buffer exchange purposes.
Figure 9. Schematic of a cross-flow filtration system. A is the system process tank containing the retentate, B is the feed stream, C is the system pump, D is the filter module, E is the permeate stream, F is the retentate stream and G is the diafiltrate stream. (Pressure gauges at the inlet and outlet of the filter module are not shown). The figure is reproduced from paper II.

The advantage of this set-up is that due to the movement of fluid over the membrane, the build-up of a filter cake is reduced. Another advantage is the possibility to perform a washing (diafiltration) of the material in order to increase yield, decrease a contaminant or just change the solvent matrix. Cross-flow filtration has found use in many industrial processes, e.g. cell harvest and protein concentration. Systems are commercially available in scales ranging from mL to m³.

In paper II, a cross-flow filtration system (Lab scale TFF system, Millipore, Sweden) equipped with an ultra-filtration filter module (Pellicon XL 1000
kD, Millipore, Sweden) has been used to gradually change the electrolyte environment of a bacterial suspension during conductivity measurements.

**Light and fluorescence microscopy**

The light microscope

The light microscope can be used to visualize, for example, cells, bacteria and other objects in the micrometer range. The first light microscope was invented by Zacharias and Hans Janssen in 1590 (Hans Lippershey is also mentioned as a parallel inventor), and led to, for example, the discovery of cells in the following century. The first light microscopes were so called bright-field microscopes, which, in principle, consist of a sample holder, a light source and a system of lenses to focus the light on the sample and to form the image.

The quality of image obtained is dependent on differences in refractive index within the sample. The contrast is strong if these differences are large and vice versa. To enhance the contrast one must either increase the differences in refractive index in the sample (by staining) or use special optical techniques such as phase contrast or differential interference contrast to amplify the differences.

For an image to be formed, the size of the sample must be comparable to, or larger, than the wavelength of the light used, since the image is formed by the optical interaction between the light and the sample. To identify two adjacent objects as separate from each other, a certain distance is needed. This distance is called the optical resolution, \( r \), and is given by the expression

\[
r = \frac{1.2 \cdot \lambda}{2 \cdot n \cdot \sin \alpha}
\]

where \( \lambda \) is the wavelength of the light used to illuminate the sample, \( n \) is the refractive index of the medium between the sample and the objective lens and \( \alpha \) is the angular aperture of the objective lens. The term \( n \cdot \sin(\alpha) \) is often referred to as the numerical aperture of the lens. The resolution is improved by either increasing the refractive index of the medium between the sample and the objective lens, by increasing the angular aperture of the objective lens.
lens or by using a shorter illumination wavelength. In practice, the resolution limit of a light microscope is about 200 – 300 nm.

The fluorescence microscope

The fluorescence microscope is used to visualize the presence, and even the position, of fluorescent molecules in a microscopic sample. The sample is illuminated with light of wavelengths that specifically excite the fluorescent molecule(s) in the sample. The emitted wavelength(s) is then passed back through the objective to reach the detector or the observer.

For successful detection of fluorescence in a sample, the background light must be as low as possible. In a standard fluorescence microscope, the exciting light illuminates deep within the sample, exciting molecules at many depth levels, while only one plane is in optical focus. However, light from out-of-focus molecules also passes through the optics and leads to a higher level of background light and, hence, lowers the limit of detection. This background can be reduced using e.g. confocal microscopy, which suffers less from this problem.

In this work, both light and fluorescence microscopy have been used to study the movement of beads in transport experiments and the presence of fluorescent molecules at bead surfaces (paper I). It is also used to follow dielectrophoretic experiments on beads and bacteria (paper III and IV) and in general microbial work (paper II, III and IV). To allow the study of bead transport on non-transparent silicon substrates, a special module was added to the fluorescence microscope that allowed white light to both illuminate the sample from above and be passed back to the objective.

**Scanning probe microscopy**

The Scanning Probe Microscope (SPM) create an image of the sample by mechanically raster-scanning a probe over it. The image maps different properties of the sample surface which, in turn, is dependent on the properties of the probe. In the Scanning Tunnelling Microscope (STM) (Binning et al., 1982) a tunnelling current is maintained between the probe and the sample. By either maintaining a constant current or a constant distance between the probe and the sample, a map is produced where the distance or the current between the sample and the probe is registered at different positions on the sample plane.
In the Atomic Force Microscope (AFM) (Binning, Quate and Gerber, 1986) the sample is scanned with a probe (tip) attached to a cantilever. Depending on the interaction between the probe and the sample, the cantilever is deflected. By reflecting a laser-beam on the cantilever, the bending can be monitored by registering the change in position of the reflected laser-beam on a photo-detector (Figure 10). The AFM can be operated in either contact or tapping mode. In contact mode, the tip is dragged over the sample, with direct contact between the probe and the sample, and thus give information on topography and friction. In tapping mode, the cantilever is oscillated at a frequency close to the resonance frequency and the tip is touching the surface only momentarily at the maximum of the oscillation. The change in amplitude is registered using the same method as for contact mode. In this mode, the impact from the tip on the surface of the sample is less harmful compared to contact mode.

In the Magnetic Force Microscope (MFM) the sample is scanned with a special probe, coated with a magnetic material, which will interact with magnetic force gradients in the sample. The resonance frequency of the cantilever is altered by the magnetic force gradient. The probe is scanned at a constant distance over the sample surface (typically less than 100 nm), which necessitates a previous tapping mode scan to determine the sample topography. The obtained image thus map the magnetic properties of the sample.
Figure 10. Principle of the AFM. The sample is placed on a sample holder (not shown in the image), whereas the probe is connected to the cantilever. A laser beam is reflected off the cantilever and hits the multipart photo-detector. As the probe interacts with the sample, the cantilever bends and/or twists, which results in a change in the beams position on the detector.

The AFM is not restricted to pure imaging, but can also be used for example to measure surface interaction forces (Senden, 2001) or to create patterns of molecules on a surface with high lateral precision (Pavlovic, Oscarsson and Quist, 2003).

In paper I, a SPM (Nanoscope™ Dimension 3100 SPM) run in MFM mode was used to investigate the magnetic properties of the elements that make up the transport lines for magnetic beads. The obtained images were used to describe the principle of the bead transport system in terms of magnetic domains in the elements.
**UV-VIS Spectrophotometry**

Electromagnetic radiation in the wavelengths of visible and ultraviolet light (about 200 – 800 nm) can interact with the outermost electrons in molecules. If the energy of the electromagnetic radiation (photon energy) exactly equals the energy needed for an electron transition to a higher energy state, the molecule will absorb the photon. Absorption of photons in an illuminated sample lowers the intensity of outgoing light as compared to the intensity of incoming light. The fraction of incident light that passes through the sample is called the transmittance ($T$), and is defined as

$$T = \frac{I}{I_0}$$

where $I_0$ is the intensity of the incoming light and $I$ is the intensity of the light that passed through the sample. Further, the transmittance is related to the absorbance ($Abs$), by the expression

$$Abs = \log\left(T^{-1}\right)$$

The absorbance of light in a sample follows Lambert-Beer’s law,

$$Abs = \varepsilon \cdot b \cdot c$$

where $b$ is the distance that the light travels in the sample (Lambert’s law), $c$ is the concentration of the light-absorbing material in the sample (Beer’s law) and $\varepsilon$ is the absorptivity constant. The absorptivity constant is material, solvent and wavelength-specific.

In this work, spectrophotometry has been used to determine both the amount of specific chemical groups (paper I) and the amount of a suspended material (bacteria) (Paper II, III and IV). In the latter case, light scattering rather than electronic transitions are responsible for the absorbance.
Surface modifications for reducing protein-surface interactions

Proteins are hetero-polymers which are crucial for all life. The more than 20 different amino acids, which are used as building blocks, can contribute both with charges and hydrophobicity depending on the chemistry of the side-groups. Acidic and basic side-groups change their charge depending on pH, thus making the overall charge of the protein pH-dependent. The sequence in which the amino-acids are connected is called the primary structure. The chain can fold into secondary structures, sheets and loops, and further into complicated 3-dimensional, tertiary, structures. Globular proteins are tertiary structures or associated tertiary structures (referred to as quaternary structures), and often have a very specific function, such as an enzyme for a biochemical reaction or as an antibody capable of recognizing a surface structure on e.g. a virus. When interacting with a surface, some proteins can undergo large structural changes whereas other are more structurally stable. Taken together, the complex and often quite flexible structure of proteins makes their interaction with surfaces a non-trivial matter. A description of all aspects of protein surface-interactions is therefore not the scope of this thesis, but a brief presentation of the forces involved in protein interactions is found in chapter 2.

Hydrophobic and electrostatic forces involved in protein–surface interactions can be reduced by modifying the surface with polymer brushes like PEO or derivatives of PEO. In addition, the presence of polymer brushes on a surface decrease the risk for conformational changes of an adsorbed protein and can serve as a lubricant between slipping surfaces (Norde, 2003). Polymer brushes are, in principle, polymer molecules which are bound to a surface at one end and the other end hanging out in the solution. As discussed in the chapter on surface forces, steric repulsion occurs when a polymer layer on a surface is compressed. The interaction between PEO-coated surfaces has been studied experimentally by Klein and Luckham (1982; 1984) and Luckham and Klein (1985). Theoretic studies on PEO-coated surfaces have been performed by de Gennes (1979; 1981; 1982; 1987). Steric repulsion between polymer coated surfaces in a good solvent is explained as an increased osmotic pressure in the vicinity between the surfaces and a decreased entropy due to less conformational freedom of the polymers when the surfaces approach. Jeon et al. (1991) modelled protein interactions on PEO-coated surfaces and concluded that long chain-length
(PEO with 80-120 monomer units) and good surface coverage are important for the surface to be protein resistant. In the same study, it was also concluded that good surface coverage of the polymer is of higher importance than the polymer chain-length for the surface to be protein resistant. In the magnetic transportation experiments described in this thesis (Paper I), PEO provided with thiol end-groups, SH-[CH₂-O-CH₂]₇₅-SH, has been used to modify the chip-surfaces. The chip-surface is coated with a thin layer of gold, as described in the following section, onto which the thiol modified PEO can form a chemical bond (Nuzzo and Allara, 1983; Nuzzo, Dubois and Allara, 1990). Figure 11 shows a simplified detail of the chemisorption process. However, the chemical details of the thiol-gold bond is not clear.

![Figure 11. Chemisorption of PEO-SH onto a gold surface.](image)

Additionally, magnetic beads with PEO coatings (-NH-(CH₂-O-CH₂)₂₀₀-NH₂) have been chosen for the same reason.

**Physical methods for surface modification**

The transport microchip described in paper I was fabricated by optical lithography. Optical lithography uses photo-resists that can be applied as thin-film coatings on surfaces where a pattern has to be created. The process has to be performed in a high-class clean room in order to avoid dust contamination. Further, the resist film must form a uniform isotropic medium, which is achieved by optimizing the spin-coating procedure with respect to spin speed. The photo-resist film is then exposed to blue or, more often, UV light behind an optical mask whose pattern is reproduced onto the resist. A negative photo-resist, as the one used here, is a photosensitive material whose molecules become cross-linked when exposed to blue or UV light. The light-energy dose has to be optimised to get a uniform cross-
linking of the exposed area. Exposed parts of the photo-resist film become insoluble to liquid developers and acquire a large chemical, mechanical and thermal resistance; on the contrary the unexposed parts remain soluble and are selectively removed when immersed into the developer. The pattern thus created in the resist define the obtained shape on the chip surface in the evaporation step. The remaining resist is finally removed and chromium (which increases adhesion of the gold to the surface) followed by gold is sputtered on the surface. Figure 12 shows a schematic of the process.

Figure 12. Sketch of an optical lithography process followed by gold-coating.

The procedure for fabricating the transport chip used in paper I is described in more detail below.

1. Spinning of ma-N420 on a silicon wafer coated with a native oxide layer on top at 5000 rpm for 30s.
2. Pre-baking on a hot-plate at 95°C for 2min 30s.
4. Development in ma-D 332 S developer for 1 min.

5. Reactive Ion Etching (RIE) based cleaning process in a plasma of oxygen and nitrogen for 20 min.

6. e-beam evaporation of 70 nm thick permalloy in an Edwards Auto 306 FL 400 evaporator with a carbon crucible filled with 1 mm thick Ni$_{80}$Fe$_{20}$ wire (GoodFellow)

7. Resist-stripping in ultrasonicated acetone for 3 min and rinsing in IPA.

8. Sputtering of 10 nm thick chromium and 10 nm thick gold.

**Protein coupling chemistry**

Reversible coupling of proteins to primary amines can be achieved by the coupling reagent N-Succinimidyld 3-(2-pyridyldithio)-propionate (SPDP) (Carlsson, Drevin and Axén, 1978). In paper I, magnetic beads provided with amino groups at the surface were reacted with SPDP to introduce the SS-pyridyl group according to Figure 13 (upper). The beads were thereafter reacted with the reducing reagent dithiothreitol (DTT) (Cleland, 1964) which chemically reduced the disulphide bond and thus introduced thiols at the bead surface according to Figure 13 (lower).
SS-pyridyl groups were introduced in proteins in the same manner as on the magnetic beads (Figure 13, upper). The side-chain of the amino-acid lysine present in the proteins contains a primary amine that can be modified in this way. Beads with thiolated surfaces were then reacted with the SS-pyridyl proteins to couple the proteins to the bead surface (Figure 14).

The proteins coupled to the beads can then be released by chemically reduction of the disulphide bond with a reducing agent such as DTT (Figure 15).
5. Summary and discussion of papers.

*Paper I*

This paper presents a system for the controlled transportation of magnetic beads, loaded with proteins, at a microchip surface. The principle of transportation is based on magnetism, and utilizes an external rotating magnetic field which in combination with a pattern of magnetic elements on the chip surface produces the driving force for the bead transport. Further, the system is made compatible with proteins by chemical modifications of the surface of both the beads and the microchip. This is also evaluated and presented in the paper.

**Principle of bead transportation**

The transport system is based on magnetization of micron-sized triangular magnetic elements patterned on a silicon chip (Figure 16). The elements are magnetized by an external rotating magnetic field. As the field rotates, the magnetization of the elements changes (Figure 16, bottom). Magnetic beads are attracted to the rim of the magnetized element and follow the magnetization as the field is rotated. At the space between neighboring elements, the bead will sense a greater attractive force from the apex of the neighboring element than from the base of the current element and, hence, make a jump to the neighboring element. This jumping continues as the field is rotated and thus the bead is transported in the direction given by the elements accordingly:

(Start at) apex → follow rim to opposing base → jump to neighboring apex → follow rim… *etc.* (Figure 16, top).
The described system is based on the work by Gunnarsson et al. (2005). In that study, a pattern of ellipses was used to facilitate the bead-transport. During the first series of experiments performed for transport of protein-coated beads the same system of ellipses was used, but was later on substituted by the triangular pattern. As compared to the ellipses, the triangles show more forward movement per turn of field rotation since there was a wigglier bead-movement with the ellipses. The system with ellipses, on the other hand, has an advantage of having a possible sorting mechanism, described in the paper by Gunnarsson et al. A combination of triangles for smooth lateral transport and ellipses for junctions/sorting is fully possible to construct. Such a system would incorporate the advantages from both structures. This, however, has not been investigated in this thesis. Structures other than triangles or ellipses are also possible to design. Important characteristics when designing new structures are size and shape, which must be chosen so to achieve multi-domain elements with minimal magnetic remanence.

Strategies for minimizing surface-related problems
The most critical factors for successful transportation of protein-covered beads have been identified as the friction and sticking forces between the bead and the surface. Gunnarsson et al. (2005) transported 2.8 μm
Dynabeads with amino-functionalized surface in the presence of a high concentration of sodium dodecyl sulphate (SDS) with the intention of eliminating sticking problems. In that investigation, the chip surfaces consisted of the silicon chip with the magnetic elements in direct contact with the bead suspension. When transporting protein-coated beads on those surfaces, there were severe problems with sticking. The beads were mostly 2.8 µm Dynabeads®, functionalized with diverse proteins. To solve the problems with sticking and future problems with conformational changes of proteins and possibly corrosion of the magnetic elements, the following strategies were used:

- Beads with a PEO-grafted surface were used in the transport experiments, since they reduce the risk for conformational changes of the protein (Norde, 2003), which otherwise would be a problem in future applications where the function of the protein is crucial, e.g. in antibody-antigen recognition.
- The chip-surface was coated with a thin layer of gold followed by a coating of PEO, described in the methods section. Since PEO is readily soluble on water, the presence of PEO on both surfaces should promote steric repulsion of the surfaces (see chapter on surface forces and methods section). The gold-layer also serves as a protecting layer for the magnetic elements to reduce the risk of corrosion.
- To further reduce sticking, a small amount of a non-ionic detergent (Tween-20, 0.5 %w/v) was added to the bead-suspension. The presence of a detergent helps preventing non-specific binding of proteins to surfaces.

The PEO-coating of the chip surfaces was investigated by x-ray photoelectron spectroscopy (XPS) and contact angle measurement.

**Model proteins and their chemical coupling to bead surfaces**

With the intention of investigating the effect of sticking discussed above, four model-proteins were selected for the transportation experiments:

- Lysozyme, a small protein with isoelectric point around 11.5.
- α-lactalbumin, a small protein with isoelectric point around 4.5.
- HSA, a medium sized protein with isoelectric point around 5.8.
- IgG, a large protein with isoelectric point between 6.8-8.5.
The proteins were chosen to cover different sizes and charges (at neutral pH), in order to further ensure the general applicability of the system. The protein α-lactalbumin is also known to have a tendency to undergo conformational changes, which is a further advantage of using it as a non-ideal (from a “sticking” perspective) model protein. For a discussion of forces between molecules and surfaces in general, see chapter 2.

A thioldisulphide exchange reaction was used to couple the proteins onto the beads (see methods section). The amount of protein per bead was 0.2 to 3 pg equivalent to about 3 to 26 attomole protein per bead, determined by amino-acid analysis. By using the thioldisulphide exchange reaction the proteins can be released from the beads by addition of a reducing agent, DTT (see methods section). To evaluate the release of the proteins on the chip surface, the beads were coated with fluorescein-labeled proteins and thus the protein release could be observed in the microscope as a disappearance of fluorescence on beads at the chip surface as DTT was added to the system. This procedure is, however, not presented in the paper. Instead, an antigen-antibody reaction was used for monitoring loading of a second molecule on the beads. The system HSA/anti-HSA was used for the purpose. The advantage of using an antigen-antibody reaction is that it also gives information on the conformational state of the protein on the beads (HSA), since the used antibody (FITC-anti-HSA) binds only to the native protein. As a negative test, beads coated with IgG was used. The presence of fluorescence on the negative test would indicate non-specific binding of the antibody to the beads. The reaction was evaluated by fluorescence microscopy.

**Results and discussion**

The presence of PEO on the chip surface was found to be of crucial importance for the transport to be successful, since severe sticking was evident in the case of non-coated chips. When both the chip surface and the particles were coated with PEO, but in absence of a detergent, some problems were still encountered to transport protein-coated beads. This indicates remaining van-der-Waals or hydrophobic interactions between the proteins on the beads and the chip surface. Due to the relative short PEO

---

5 In amino-acid analysis, the protein is hydrolyzed into its constituent amino-acids, followed by separation and quantification of the amino acids, usually by high performance liquid chromatography (HPLC).
chain length on the chip, the effect of steric repulsion may be too small. Another explanation is that the surface coverage of PEO may be incomplete (Norde, 2003; de Gennes, 1979). The use of beads without PEO (in combination with PEO on the chip) gave roughly the same transportation performance as for beads with PEO (see paper for details on evaluating the bead mobility). For transportation purposes only, it is therefore not necessary to have PEO on the beads. However, there is still an important advantage of using beads with a PEO surface, since it reduces the risk of conformational changes of proteins and non-specific binding of other proteins to the bead. The presence of non-specific binding of proteins to beads without a PEO-coated surface is also demonstrated in the paper.

If related to the work by others, see introduction, the main advantage of the presented system is its relative simplicity (e.g. absence of micro-channels and electrical wiring) in combination with the possibility of controlling the position of single beads at a chip surface with micrometer precision. The main progress of this work if related to the original system presented by Gunnarsson et al. (2005), is that due to the efforts on surface modifications, controlled transport of proteins in the native state is now possible with the system.

**Paper II**

This paper describes a method for determining the conductivity of bacteria in suspension. As described in the introductory theory of dielectrophoresis in chapter 3, conductivity is important for the dielectrophoretic behaviour of particles at low frequencies. If conductive particles, such as bacteria, are present in a suspension, they affect the total conductivity of the suspension (van der Wal et al., 1997). By varying the conductivity of the media in which the bacteria are suspended and measuring the difference in conductivity between the media and the suspension, the isoconductance point can be determined. At this point, the bacteria doesn't contribute to the overall conductivity of the suspension. They are, so to say, invisible from a conductivity point of view. Furthermore, the isoconductive point is independent of the volume fraction of bacteria in the suspension, a factor which otherwise can be very difficult to determine exactly (Yunus et al., 2002; van der Wal et al., 1997).

It was decided to use cross-flow filtration as an alternative to centrifugation-resuspension, which is the common method for varying the conductivity.
during conductivity measurements on bacterial suspensions (Yunus et al., 2002; van der Wal et al., 1997; Markx et al., 1994).

The results in this article on the conductivity of Bacillus subtilis (7000±1500 µS/cm) differ from previously published data (935±96 µS/cm) (Markx et al., 1994) by being almost an order of a magnitude higher. This is interesting since the cross-flow filtration approach differs from the centrifugation-resuspension approach in the following way: With the cross-flow filtration method, the conductivity is gradually changed from high to low conductivity by the diafiltration procedure, whereas in other published methods the conductivity is changed from low to high conductivity by starting the measurement with bacteria previously washed several times in low conductivity media (sometimes deionized water) and increasing the conductivity by adding aliquots of salt solution. Furthermore, with the cross-flow filtration method the suspension and the suspending media are continuously separated at an equilibrium state, i.e. the measurement is made when the bulk conductivity ceases to change after a change in conductivity.

From the results in paper II the conclusion was drawn that the treatment of the cells during conductivity measurements seems to be of importance. An explanation of our high conductivity value is very uncertain since the available data set is too small. However, some further discussion of the issue, not taken up in the paper, is made below.

The cell interior may not be depleted of ions to the same extent as if compared to the centrifugation-resuspension approach so ions can still be transported out to the surface of the cell, resulting in a higher measured conductivity. Note that it is assumed that the ions remain at the surface of the bacteria, due to the equilibria discussed above. The results in paper II can be compared with the work by Markx et al. (1996) in which with dielectrophoretic separation of bacteria with conductivity gradients was studied. The authors found that bacteria were released from the electrodes at much lower conductivity than expected, e.g. Micrococcus luteus released already at 40 µS/cm but the conductivity value of the bacteria was stated as 1557 µS/cm and should therefore release close to this value, since at this point the Clausius-Mossotti factor will become zero. The author noted the discrepancy but could at that time give no explanation to this. The leakage of ions from the bacteria could explain this phenomenon, as it would cause a local high conductivity around the bacteria which in turn would cause them to release from the microelectrodes despite the low bulk conductivity.
In conclusion, more work needs to be performed on other microorganisms to strengthen the general applicability of the method. Another important task is to perform a hysteresis loop where the conductivity is changed below the isoconductance point and back again. This will give a hysteresis curve, which would be very valuable since the presence (or absence) of hysteresis can strengthen (or weaken) the findings about the discrepancy in $B.\ subtilis$ conductivity. Desirable, although not necessary, would be a direct differential measurement with two separate probes, one in the process tank and one in the permeate stream.

**Paper III**

In this paper the dielectrophoretic response of *Escherichia coli* was studied. The overall aim of the project was to investigate if sub-populations of a certain bacterial species could be identified and eventually separated with dielectrophoresis. A device capable of identifying and/or separating sub-populations of bacteria could be of use in e.g. fermentation technology, where it has been shown that recombinant bacteria may lose the plasmid coding for the recombinant protein and thus allowing for a part of the population to replicate without producing the recombinant protein (Corchero and Villaverde, 1998).

The dielectrophoretic response of the bacteria was studied in devices containing micro-electrode structures (figure 2 in the paper). The micro-electrodes were connected to a function generator and an oscilloscope (Figure 17). A fluorescence microscope was used to study the dielectrophoretic response of the bacteria.
The studied bacteria were a strain of *E. coli* capable of producing protein aggregates, inclusion bodies (Pulkkinen, 2003). Since inclusion bodies are quite large objects contained within the bacteria, some hundred nanometers, focus was placed upon the separation of bacteria containing inclusion bodies from bacteria without such inclusion bodies.

In the paper the preliminary experiments on the dielectrophoretic response of the bacteria are presented. The bacteria were trapped in between the electrodes at 10 kHz to 10 MHz and 10 V peak-to-peak (figure 4 in the paper). The bacteria aligned with the field at frequencies up to 10 MHz (figure 4 a and b in the paper). At increased frequencies, the bacteria aligned perpendicular to the field (figure 4 c in the paper). This type of behavior is common for ellipsoidal particles and is explained in the following way: dipoles tend to align with the field (see chapter 2). When the frequency is low enough, the dipole can form along the longest axis of the ellipsoid, and thus place the partial charges as far apart as possible (and closest to the opposing charges at the electrodes). At high enough frequencies, the dipole can no longer form along the long axis, due to the short time available for the charges to separate, and are instead formed along the short axis. The bacteria then align with the short axis along the field. Another phenomenon which was observed was the formation of pearl-chains, as a result of a dipole-dipole interaction between similar particles. Examples of pearl-chains are found in figure 4 in the paper. Rotation of the particles at the electrode
edges was also observed. An explanation of this can be the presence of an electro-osmotic flow at the electrodes (Morgan and Green, 2003), but the phenomenon was not studied further. Negative dielectrophoresis was not showed with this device.

A prototype fluidic device (figure 3 in the paper) for dielectrophoretic pulling of bacteria between two parallel laminar flows was also presented. The flow properties were investigated (not shown in the paper) by injecting colored water in one of the channels and pure water into the other channel. It was found that the streams were not mixed during the length of the channel (see detail on fluid channel junction in Figure 18). The fluid velocity was less than 10 mm/s. With a channel cross section of 100x40 µm² this corresponds to a volumetric flow less than 2.5 µL/min and a Reynolds number below 1, which is well within the laminar-flow region, as expected for a microfluidic system (Squires and Quake, 2005). Different strategies for pumping were also evaluated: SAGE instruments syringe pump model 351 equipped with a Hamilton Microliter #802 syringe gave stable flows between 0.5 and 2.5 µL/min. Gravity flow pumping inspired by a U-tube manometer delivering stable flows well below 1 µL/min was also tested.

![Figure 18. Detail on fluid channel exit junction. The upper stream is blue colored water and the lower stream is milli-Q water. Flow direction is from right to left.](image)

A suspension of *E. coli* was repeatedly washed, resuspended in milli-Q and finally injected into the DEP-device. Dielectrophoretic capture at different frequencies and potentials (peak-to peak) of the applied field was tested. It was found that the bacteria were collected at the pointed electrodes at frequencies above 200 kHz and 10-15 V peak-to peak. In this experiment, a function generator capable of maximum 5 MHz/20 V peak-to peak was used.
(BK-Precision 4011). In Figure 19 trapping of acridin-orange stained \textit{E. coli} in a micro-fluidic channel is demonstrated. The applied field is 5 MHz at 10 V peak-to-peak and the fluid velocity below 100 µm/s. The planar counter electrode is situated below the pointed electrodes just outside the lower part of the figure. It can be noted that the acridin-orange staining of the bacteria may influence the dielectrophoretic response, and is only used to enhance the visualization of the bacteria in the fluid stream.

![Figure 19. Trapping of fluorescent labelled \textit{E. coli} at 5 MHz, 10 V peak-to-peak at pointed electrodes situated within a micro-fluidic channel. The counter-electrode is situated just outside the lower part of the picture. Flow direction is from right to left (fluid velocity is below 100 µm/s). Figure presented in paper III.](image)

In conclusion, basic dielectrophoretic studies of \textit{E. coli} in micro-devices were undertaken and showed trapping of \textit{E. coli} from a fluid flow in a micro-fluidic channel.

During the continued work, not shown in the paper, the separation of bacteria containing inclusion bodies from normal bacteria was not achieved (which was the overall aim of this study). The qualitative methods for evaluating the dielectrophoretic response, which were available at that time, were insufficient to conclusively state if this was indeed possible. Basically, the dielectrophoretic collection of \textit{E. coli} differing in inclusion body content was studied at different frequencies. The production of the 33 kD protein, building up the inclusion bodies in the bacteria had been verified by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and by microscopy. To prevent bias due to prior knowledge of which samples contained bacteria with inclusion bodies, the samples were double blinded \textit{i.e.} one person randomly marked the samples and a second person randomly re-marked them before the dielectrophoresis experiments. Furthermore, each sample was duplicated. When the results from the dielectrophoresis experiments were finished, the markings were translated.
dielectrophoretic collection at various frequencies differed to the same extent between samples as between replicates, and it was therefore concluded that more fine-tuned methods must be used if small differences in electrical properties are to be used for separation. Recently, Sarrafzadeh et al. (2005) showed that the formation of structures such as spores within the bacterial cytoplasm influences the permittivity of the bacteria and can be measured with on-line permittivity probes. Castellarnau et al. (2006) used dielectrophoretic cross-over measurements to distinguish mutants of *E. coli* from the wild-type. These recent findings strengthen the idea that a dielectrophoresis-based separation of bacteria differing in structures within the bacterial cytoplasm may be possible. During cross-over measurements, the dielectrophoretic response of the particle is observed during variation of both frequency and medium conductivity. At low frequencies and medium conductivities, the particle typically show positive dielectrophoresis and thus by increasing the frequency the particle will show zero dielectrophoretic force at a certain frequency (the cross-over frequency) and negative dielectrophoresis above that frequency. The procedure is repeated at different conductivities and thus a cross-over frequency vs. conductivity graph can be produced (Morgan and Green, 2003).

**Paper IV**

Paper IV is a continuation of paper III. In this paper, straight interdigitated electrodes were used for the spatial separation of different sized beads and point electrodes similar to those used in paper III for the spatial separation of *E. coli* from *Saccharomyces cervisae*. In paper III it was not possible to show negative dielectrophoresis, so an important experiment was to show this for a simple system such as different sized polystyrene beads. The separation was based on the selection of frequencies to achieve differences in positive and negative dielectrophoresis between different beads. In figure 4b in paper IV is showed a spatial separation of 0.5 and 2 µm polystyrene beads occurring at 8 µS/cm, 1.4 MHz and 10 V peak-to-peak, where the 2µm beads showed negative dielectrophoresis and the 0.5 µm beads showed positive dielectrophoresis. At higher frequencies (figure 4c in paper IV) both bead-types showed negative dielectrophoresis. At lower frequencies (figure 4a in paper IV), both bead-types showed positive dielectrophoresis.
Another important experiment was to demonstrate the spatial separation of different types of cells. The separation of *E. coli* from *S. cervisae* was chosen because of their relative large difference in size. Separation was achieved by choosing a frequency where both organisms collect at the electrodes by positive dielectrophoresis, but apply a voltage low enough to ensure that only the larger cell is collected. In figure 6a in paper IV the yeast cells were collected between the electrode points whereas the bacteria was unaffected by the field due to their smaller size. In Figure 20 (not shown in the paper) a similar spatial separation is demonstrated. In this figure, the random orientation of the bacteria is clearer than the figure in the manuscript. The conductivity of the medium is very low to ensure positive dielectrophoresis.

Also demonstrated in the paper is a preliminary test of microelectrodes for on-chip conductivity-measurements of bacterial suspensions. That work was continued outside of this project, where it was shown to be useful to study the release of ions from bacteria when killed by heat-shock (Jönsson *et al.*, 2006).

Figure 20. Spatial Dielectrophoretic separation of *Saccharomyces cervisae* and *E. coli* at 20 MHz and 1 V peak-to-peak. The yeast cells is collected in the gap
between the electrode points, whereas the bacterial cells are unaffected by the field.

In conclusion, this paper presented some continued studies from paper III on dielectrophoresis as a tool for manipulating microparticles, in particular microorganisms. Spatial separation of polystyrene beads differing in size was shown. Further, selective positioning of *S. cervisae* between pointed electrodes, in a mixture of *S. cervisae* and *E. coli*, was shown. Finally, preliminary experiments on conductivity measurements of *E. coli* with microelectrodes were shown. Since a clear demonstration of both positive and negative dielectrophoresis was possible to achieve with the device used in this paper, cross-over measurements such as those demonstrated by Castellarnau *et al.* (2006) may also be feasible. This is, however, yet to be tested.
Svensk populärvetenskaplig sammanfattning

Sedan mikroskopet uppfanns under 1600-talet har vi kunnat se och lära oss allt mer om den för oss annars osynliga mikrovärlden. Enkla strukturer som ler-mikropartiklar likväl som levande material som celler och bakterier tillhör denna mikrovärld, där de fysikaliska förutsättningarna ter sig helt annorlunda än de gör för oss människor. Ytegenskaper är en viktig parameter att ha kontroll på när man vill försöka förstå de förutsättningar som gäller på mikronivå, eftersom andelen yta hos partikeln ökar relativt sett allt eftersom partikels storlek minskar. Storleken har en annan avgörande roll om vi skulle vilja aktivt flytta runt på enstaka föremål i mikrometerstorlek. Det är helt enkelt väldigt svårt få tag på och kontrollera mikropartiklar med de metoder som vi normalt brukar använda i vår makrovärld. Metoder för att kunna kontrollera mikropartiklar är viktiga, eftersom deras yta kan användas som bärare av molekyler och alltså kan möjliggöra studier av friktion och nötning på ytor där man har för avsikt att förstå och utveckla nya material eller för positionering av molekyler med stor precision. Principen är generell och kan användas i olika applikationer där man t ex vill fånga upp en molekyl på en viss position på ett mikrochip och transportera den till andra positioner på exempelvis mikrochipet. Tänkbara applikationer kan vara instrument för kemisk analys, diagnostik men framförallt för materialkaraktärisering och utveckling av nya material.

Acknowledgments

The first thanks goes to former and present members of Sven Oscarssons group and the collaborators at the Ångström laboratory. The experience I got from working with you during the last years of my time as a PhD-student is invaluable. The second thanks goes to the collaborators in the DEP-project for all the hard and frustrating work in an area which was new to all of us but well known to others (as we found out the hard way). We did quite well after all, and it was kind of fun from time to time.

Colleagues and students at former IBK in Eskilstuna: wish you all the best.

Släktingar i Sverige och Norge.

Janne.

Magnus och Stanley.

Till sist ett stort tack till min älskade familj:
Susanne, BjörnErik och Cornelia.
References


Swarco Vestglas, Vestische Strahl- und Reflexglas GmbH, Recklinghausen, Germany.


