An integrated microfluidic chip with continuous flow centrifugation for sample concentration, particle collection and particle transport

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**AN INTEGRATED MICROFLUIDIC CHIP WITH CONTINUOUS
FLOW CENTRIFUGATION FOR SAMPLE CONCENTRATION,
PARTICLE COLLECTION AND PARTICLE TRANSPORT**

SUN HUI

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ABSTRACT

Biological sample concentration in a large volume is essential to bio-analyses of environmental samples. If particles in drinking water can be high-throughput concentrated, collected and transported into a bio-analysis system easily, an automatic online monitoring system of drinking water can be developed. The present work aims to design and fabricate a chip with single channel to achieve continuous flow centrifugation of particles in water sample with integrated capability of droplet based particle collection and particle transport to downstream functional modules on the same chip, such as a cell sorter or a PCR system. Two methods of integrated continuous flow centrifugation, namely the Point Capture method and the Distributed Surface Capture method, have been proposed and investigated. A J-channel chip is designed for the application of the Point Capture method, and a spiral channel chip is designed for the application of the Distributed Surface Capture method. For fluorescent bead suspension, our prototype of the J-channel chip is able to achieve a concentrating efficiency ranged between 50% and 93% with a trend for higher concentrating efficiency at a lower feed rate (feed rate between 5-20 mL/h). Our spiral channel chip is able to achieve a concentrating efficiency ranged between 50% and 100% with a trend for higher concentrating efficiency with a lower feed rate (feed rate between 60-180 mL/h). The continuous concentration and collection of *E. coli* cells in the J-channel chip are also demonstrated. The integration between the sample concentration, the droplet based particle

collection and downstream functional modules such as micro-reactors has also been achieved.

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Chapter 1 Introduction

1.1. Background

Infectious diseases caused by pathogenic bacteria, viruses, and protozoa are the most common and are widely associated with contaminated water supplies. A primary concern in monitoring the quality of water and wastewater is the presence of microbial pathogens that pose threats to drinking-water supplies, recreational waters, source waters for agriculture and aquaculture, and aquatic ecosystems. The control of microbiological hazards to reduce the incidence of infectious disease, improve the quality of water, requires rapid and simultaneous detection of multiple microbial pathogens including bacteria, viruses, and protozoa. In addition, simultaneous detection of sub-strains of water-borne bacterial and viral pathogens is desired. Traditionally, the detection of water-borne pathogen was restrained by the ability to culture these organisms in a general growth medium. However, majority of bacterial population cannot be cultivated on a general-purpose media. Moreover, culture-dependent techniques select for fast-growing heterotrophy and therefore the cultivable strains do not adequately represent the composition and vast diversity of water-borne pathogens. Recently, with advances in PCR methods and microarray assays, both water-borne pathogen community composition and quantization of pathogen load is being realized. The high sensitivity and specificity of molecular techniques, allows direct detection of pathogens in water samples.

The nature and form of drinking-water standards may vary among countries and regions. There is no single approach that is universally applicable. It is essential in the development and implementation of standards that the current and planned legislation relating to water, health and local government are taken into account and that the capacity to develop and implement regulations is assessed. The basic and essential requirements to ensure the safety of drinking-water are a “framework” for safe drinking-water, comprising health-based targets established by a competent health authority; adequate and properly managed systems (adequate infrastructure, proper monitoring and effective planning and management); and a system of independent surveillance. Infectious diseases caused by pathogenic bacteria, viruses and parasites (e.g., protozoa and helminthes) are the most common and widespread health risk associated with drinking-water [1]

The pathogens that may be transmitted through contaminated drinking-water are diverse. The current classical methods approved for monitoring quality of water are time consuming, labor intensive and presents serious limitations from both qualitative and quantitative points of view. Thus to circumvent these lengthy methods there is an urgent need for rapid, cost effective and easy to use method for detecting and quantifying waterborne pathogens. As environmental samples often have the properties of large volumes with low cells or bio-molecules concentration, it is essential to concentrate the particles from large volumes into small volumes. Various methods can be used in large volume concentration, such as centrifugation, dielectrophoresis, filtration, etc. However, most of them

have low throughput limitations. Thus it is necessary to develop a method to concentrate particles at a high throughput with high efficiency.

1.2. Objectives

The goal of this research is to design an integrated chip which can concentrate biological particles by continuous flow centrifugation, collect the particles into a DI-water droplet, and transport the particles to downstream functional modules, such as a cell sorter or a PCR system. The following tasks need to be accomplished:

- 1) Design and fabricate a chip with a channel used in continuous flow centrifugation with integrated droplet based particle collection and integrated particle transport to downstream functional module, such as a cell sorter and a PCR system.
- 2) Carry out the experiments to investigate the relationship between the particle concentrating efficiency and the feed rate.
- 3) Treat the surface of the channel wall in the chip to promote the bacterial resistant, so that the concentrated particles can be collected easily.

1.3. Thesis organization

This thesis covers a preliminary research on the basic mechanisms of various concentration methods and their applications. This section describes the entire structure of this thesis. Chapter One, the introduction of this thesis, describes

the motivations and the objectives for the project. Chapter Two is divided into two parts. The first part is the literature review of modern concentration methods, including the advantages and disadvantages of each method. The second part is to introduce the dominating factors for decreasing the bacterial adhesion. In Chapter Three, not only the reason for using current continuous flow centrifugation, but also the experiments including the chip fabrication process, particle preparation, and instruments will be discussed. In Chapter Four, the results of experiments will be presented and discussed. In Chapter Five, a conclusion to summarize the project work will be presented. In Chapter Six, improvement and future work will be proposed to improve the chip design and experiments.

Chapter 2 Literature review

2.1. Sample concentration

2.1.1. Introduction to sample concentration

Real-world biological samples are often in relatively large volumes, which results in low concentration of cells and bio-molecules in the sample, or small volumes containing rare cells, bio-molecules, and pathogens like bacteria and virus. Hence, the concentration of target cell/analyte is low, which leads to the inaccuracy of the sample detection by analyzing an aliquot. The formidable challenge for any sample concentration method to accept large volumes or small volumes of raw samples, perform sample concentration, extract the concentrated sample and load it into a small volume of carrier fluid (volume reduction), and transport the small volume of concentrated sample to an analytical station (the function of cell separation, cell lysis, DNA purification, and PCR) for characterization/diagnostics, still persists. Now various methods such as centrifugation [2], fluorescence activated cell sorting (FACS) [3], magnetic activated cell sorting (MACS) [4], filter-based sorting, capillary electrophoresis and dielectrophoresis [5] have been reported in literature to enrich/concentrate bio-molecules and cells.

2.1.2. Methods for sample concentration

2.1.2.1. Conventional centrifugation

Centrifugation is to separate mixtures by the use of centrifugal forces. For the working principle of centrifugal force, the more dense components run away from the axis, while the less dense components run towards the axis. Centrifuge is a piece of equipment that applies the principles of centrifugation for particle separation, clarification, thickening or concentration [6].

It is the centrifugal force that drives different components to certain positions, thus it is necessary to know more about centrifugal force. Centrifugal force depends on the angular speed and the distances between axis and components.

The centrifugation force of every kilogram can be calculated by

$$\frac{F_c}{m} = \omega^2 r = \left(\frac{n \times 2\pi}{60}\right)^2 r = 0.011n^2 r \quad (2.1)$$

where F_c is the centrifugal force, m is the mass of particles, and ω , n , r are angular velocity, the rotating speed of the rotor, and the distance of particles from the center of rotation.

The speed of rotor is a significant component to classify the centrifuges. The speed in different levels can lead to the diverse properties and applications of the centrifuges. Table 2.1 summarizes the properties and applications of low-speed, high-speed and ultracentrifuges [7].

Table 2.1: Properties and applications of centrifuges [8].

	Class of centrifuge		
	Low-speed	High-speed	Ultra-centrifuge
Speed ranges(r.p.m. $\times 10^3$)	2-6	18-22	35-120
Maximum centrifugal force ($\times 10^3$)	8	60	700
Refrigeration	Some	Yes	Yes
Vacuum system	None	Some	Yes
Acceleration/braking	Some	Variable	Variable
Pelleting applicatons	-		
Bacteria	-	Yes	(Yes)
Animal and plant cells	Yes	Yes	(Yes)
Nuclei	Yes	Yes	(Yes)
Precipitates	Some	Most	(Yes)
Membrane organelles	Some	Yes	Yes
Membrane fractions	Some	Some	Yes
Ribosomes/polysomes	-	-	Yes
Viruses	-	Most	Yes

where “(Yes)” represents can be done but not usually used for this purpose.

Conventional centrifugation usually takes the steps as following:

- 1) Load the rotor with sample,
- 2) Accelerate to operating speed,
- 3) Run for a specific period of time,
- 4) Decelerate to a stop,
- 5) Unload the sample.

These steps should be repeated before having processed the entire sample. If the sample processed is in large quantity, low sedimentation rate, long acceleration/deceleration time, and small capacity, the conventional centrifugation will be time consuming [8].

2.1.2.2. Filtration

Filtration is a method which applies filter to separate components from mixtures by mechanical ways, without any phase change (such as melting or evaporation)[9].

Two main applications in filtration exist:

- 1) To remove impurities.
- 2) To collect and recover valuable components from the mixture.

The first (can be called 'clarification') usually applies fine porous media to remove impurities as much as possible, while the second ('harvesting') aims for recovering the components wanted as much as possible. In our case, the second ('harvesting') aim is what we are interested in.

Generally speaking, the filtration is based on the particle concentration by filter medium, which means the particles are removed from the fluid. If categorized more specifically, the mechanisms of the filters can be divided into surface straining, depth staining, depth filtration, and cake filtration [9].

The specific descriptions of filtration mechanisms are as follows:

1) Surface straining

The particle which is larger than the pores of medium stays at the surface, and at the same time, the smaller one can pass through the medium. Figure 2.1 shows the process of filtration [9].

Figure 2.1 Filtration by surface straining [9].

This is the main operating mechanism for bar screens, and plain woven monofilament mesh. It also plays a major role in filtration with membranes.

2) Depth staining

For media which are relatively thicker than the pore diameters, particles will travel along the pore until they reach the position, where the pore diameters are too small to pass through particles. Then the particles are trapped. Felts and other non-woven fabrics utilize this mechanism, which is illustrated in Figure 2.2 [9].

Figure 2.2 Filtration by depth straining [9].

3) Depth filtration.

Although the particles are much smaller than the pore diameters, the particles can also be trapped by the pore wall or another particle already held to the wall. That's because when particles are first brought into contact to or very close to the pore wall by inertial or hydraulic forces, or by Brownian motion, they will be attached to the pore wall by Van Der Waals and other surface forces. The magnitude and efficacy of these forces may be affected by changes in factors such as the concentrations and species of ions in an aqueous solution, or the humidity of a gas. This mechanism is illustrated in Figure 2.3, and is important

for most media, but especially for high-efficiency air filters and in deep bed (sand) filters [9].

Figure 2.3 Depth filtration mechanism [9].

4) Cake Filtration.

A thick layer or cake of particles accumulates on the surface of the medium, and then acts like the filter medium for following filtration. If particles are larger than the pores, then it is surface straining before cake filtration. While if particles are smaller than the pores (down even to about one-eighth of the pore diameter), especially if the solid concentration is relatively high, a bridge can be generated across the entrance to a pore, as shown in, Figure 2.4 to form a base upon which the cake will then grow [9].

Figure 2.4 Cake filtration mechanism [9].

The actual mechanism or combination of mechanisms pertaining in any specific instance is depending on the characteristics both of the medium and of the suspension being filtered. Table 2.2 shows the relationships between the four basic mechanisms and the two broad categories of practical filtration (clarification and harvesting).

Table 2.2: The role of different filtration mechanisms in practical filtration.

Mechanism	Harvesting	Clarification
Surface straining	Major	Minor
Depth straining	-	Major
Depth filtration	-	Major
Cake filtration	Major	Minor

where, “-“ represents Normally not used, “Major” represents a major role of practical filtration and “Minor” represents a minor role of practical filtration.

In the process of filtration, several specific properties can control the capability to undertake the filtration, which include smallest particle retained, retention efficiency, resistance to flow, tendency to blind, and cake discharge characteristics. The following is to introduce each filtration-specific property:

1) Smallest particle retained

It is one of the most important properties to know the smallest particle retained.

The smallest particle retained of main media type is listed in Table 2.3 [9].

Table 2.3: Summary of the smallest particle retained of main media type [9].

Main media type	Subdivisions	Smallest particle retained (~µm)
Solid fabrication	Flat, wedge-wire screen	100
	Wire-wound tubes	10
	Edge-type	10
	Stacked discs	5
Metal sheet	Perforated	20
	Sintered woven wire	1
	Unbonded mesh	5
Rigid porous media	Ceramics and stoneware	1
	Sintered metal powder or fibre	1

	Carbon	1
	Sintered plastic powder or fibre	< 1
Cartridge	Yarn wound	5
	Bonded granule or fibre	1
	Pleated sheet	< 1
Plastic sheet	Perforated	10
	Sintered woven filament	5
	Woven mono- or multi filament (Membrane)	10
Membrane	Ceramic	< 0.1
	Metallic	< 0.1
	Polymeric	< 0.1
Woven media	Staple fibre yarn (polymeric filament)	5
Non-woven media	Dry-laid (felts)	10
	Wet-laid (papers)	2
	Wet-laid (sheets)	0.5
	Special polymeric(spun	0.1

	bonds, etc.)	
Loose media	Fibres	1
	Powders	< 0.1

2) Retention efficiency

Retained particles divided by total particles is the retention efficiency of filtration, which is also an important property for filter selection. Particle size is the main parameter to determine retention efficiency. Moreover, retention efficiency is also related to the structure of filter media, particle shape, and filtration mechanisms.

3) Resistance to flow

The resistance to flow of a filter medium depends both upon the size of the individual pores and on the number of pores per unit area. The ideal medium would comprise a mass of holes divided by the thinnest possible walls, thus presenting the maximum open area through which fluid can flow. In practice, the holes account for only a relatively small part of the surface, the exact proportion depending on the properties of the material from which the medium is made and the manufacturing process used. Large differences in resistance to flow exist among the diverse ranges of available media.

The actual resistance to flow of a fluid passing through a clean medium depends on a combination of the porosity of the medium material (i.e. the physical

structure of the pores and surrounding material) and the permeability of the medium to the appropriate fluid (i.e. the ease or otherwise with which that fluid flows through the medium).

4) Tendency to blind

Unless the filter medium (or cartridge) is to be discarded at the end of a complete cycle, it is important that the accumulated solids be easily removed by a suitable cleaning procedure, whether the operation involves clarification or solids recovery by cake filtration. A medium is said to be blinded when cleaning fails to remove residual solids, which adheres to or embedded in it, so that its resistance to flow remains unacceptably high.

5) Cake discharge characteristics

Cake discharge characteristics are of particular importance with filter cloths used in conjunction with continuous filters such as rotary vacuum drum and disc filters, where successful continuous operation is highly dependent on the completeness with which the cake is automatically removed from the working surface. The ability of a medium to discharge its cake depends very much upon the smoothness of the surface upon which the cake is residing, and hence upon the amount of fibrous material extending from the surface into the cake.

2.1.2.3. Dielectrophoresis

If a dielectric particle is suspended in an electric field, it will be polarized; the magnitude and direction of this induced dipole depends on the frequency and

magnitude of the applied electric field, and the dielectric properties of particle and medium [10,11].

Figure 2.5 Two different particles in a non-uniform electric field.

If the electric field is uniform, then the Coulombic attraction between the dipolar charges and the electric field is equal and opposite and there is no net movement, unless the particle carries a net charge and the field frequency is equal to, or near, zero. However, if the field is spatially non-uniform, then the magnitude of the Coulombic forces on either side of the particle will be different, as shown in Figure 2.5. The total electric force acting on a particle of net charge in a non-uniform field E is:

$$F = QE + \delta qE(r_+) - \delta qE(r_-) \quad (2.2)$$

If the net charge of the particle is zero ($Q=0$), the particle on the left is more polarizable than the surrounding medium and is attracted towards the strong field at the pin electrode, while the particle of low polarizability on the right is directed away from the strong field region.

The equation (2.2) can be transformed as follows:

$$F = QE + \delta qE(r_+) - \delta qE(r_-) = QE + (m \cdot \nabla)E \quad (2.3)$$

∇ is the Del vector operator and m is the induced dipole moment.

The induced dipole moment $m(t)$ for a particle subjected to a root mean square (RMS) electric field of magnitude E with three spatial components in the x, y, z directions is given by

$$m(t) = m_x(t)a_x + m_y(t)a_y + m_z(t)a_z \quad (2.4)$$

where a_x, a_y, a_z represent the unit vectors in three Cartesian coordinates.

Taking $m_x(t)$ as an example, the components of the dipole moment take the form,

$$m_x(t) = 4\pi_m r^3 [\cos(\omega t + \varphi_x) \text{Re}[K(\omega)] - \sin(\omega t + \varphi_x) \text{Im}[K(\omega)]]E_{x0} \quad (2.5)$$

where r is the radius of the particle and ϵ_m is the absolute permittivity of the suspending medium. The terms (Re and Im) represent the real and imaginary components, respectively. $K(\omega)$, the Clausius-Mossotti factor, is given by the equation:

$$K(\omega) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad (2.6)$$

where ϵ_m^* and ϵ_p^* represent the complex permittivity of the medium and particle respectively, and $\epsilon_m = \epsilon_m - j\sigma/\omega$ with σ the conductivity, ϵ the permittivity and ω the angular frequency of the applied electric field. When this dipole is induced

by a locally non-uniform electric field, the net, time-averaged dielectrophoretic force on the particle is given by the expression [12]:

$$F = (m \nabla) E = 2\pi\epsilon_m r^3 [\text{Re}[K(\omega)] \nabla E^2 + E_{x0}^2 \varphi_x + E_{y0}^2 \varphi_y + E_{z0}^2 \varphi_z] \quad (2.7)$$

where r is the particle radius, ϵ_m is the permittivity of the suspending medium, ∇ is the Del vector (gradient) operator, E_{x0} , E_{y0} and E_{z0} represent the local electric field vectors and φ_x , φ_y , and φ_z represent the local phase vectors.

When in a system the electrical field is energized by two electrodes powered by sinusoids in antiphase, the phase variation will be eliminated. Equation (2.7) can be reduced to the “classical” expression of dielectrophoretic force [11]:

$$F_{DEP} = 2\pi r^3 \epsilon_m \text{Re}[K(\omega)] \nabla E^2 \quad (2.8)$$

From equations (2.7) and (2.8), it can be seen that the dielectrophoretic force depends on the particle size (and shape) and on the magnitude and degree of non-uniformity of the applied electric field. Furthermore, the polarity of this force depends on the polarity of the induced dipole moment, which in turn is determined by the conductivity and permittivity of the particle and its suspending medium.

Conrad D James and his coworkers demonstrated the availability of highly efficient microparticle preconcentration using ac DEP and an accompanying phase separation [13].

The group of Carlos de la [14] presents an integrated micro-fluidic system for combined manipulation, pre-concentration, and lysis of samples containing *Bordetella pertussis* by dielectrophoresis and electroporation. The microfluidic device was able to pre-concentrate the amount of *B. pertussis* cells present in 200 μl of a *B. pertussis* suspension stock into a 20 μl volume. The device exhibited optimal sample pre-concentration of $6.7\times$ at a stock value of 10^3cfu/ml and at a flow rate of $250\mu\text{l/h}$.

2.1.2.4. Fluorescence-activated cell sorting

Fluorescence-activated cell sorting is a specialized type of flow cytometry. It provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell. It is a useful scientific instrument as it provides fast, objective and quantitative recording of fluorescent signals from individual cells as well as physical separation of cells of particular interest [15].

Figure 2.6 Flow chart of fluorescence-activated cell sorting [16].

As illustrated in Figure 2.6, at first load cells in the sample tube (A), and then push them into a sorting nozzle (B). At the same time, sheath fluid is pushed into the nozzle at a relative lower pressure (C). Due to the nozzle shape, the

sheath fluid develops a laminar flow (D). Cells join with the sheath stream by flowing through a needle in the center of the nozzle (E). The sorting stream flows to the laser interrogation point (F). When cells go through the laser, deflection, either refraction (Forward Angle Scatter) or reflection (Side Angle Scatter) will happen. The laser can also excite dyes and fluorophores used in cells preparation (G). All the information will be collected by the optic system (H), converted into electrical pulse, and sorting signals will be generated. A piezoelectric crystal on top of the sorting nozzle will oscillate at a certain frequency to cause the sheath stream breaking into droplets (I). The droplets will be charged (either positive or negative depending on sorting decision) by an electric pulse (J). Then, the droplets can be deflected and sorted by electromagnetic plate (K) [16].

2.1.2.5. Capillary electrophoresis

Capillary electrophoresis is a relatively recent separation method, which has become more and more important over the last decade. Although the electrophoresis has been an invaluable analytical tool for research, most of them suffer from the problem of low separation speed according to the limitation of joule heating. Most electrophoresis can only be used to low potential electrical fields for the relatively poor dissipation of Joule heat in these separation systems. As to capillary electrophoresis, capillaries are used as an electromigration channels for separation of a diverse array of analytes. The efficient dissipation of Joule heat generated from large applied fields can be achieved by the high

surface-to-volume ratio of capillaries with these dimensions. Capillary electrophoresis includes several modes, such as capillary zone electrophoresis, micellar electrokinetic capillary chromatography, capillary isoelectric focusing, capillary gel electrophoresis, and capillary isotachopheresis [17].

Figure 2.7 General schematic of a capillary zone electrophoresis instrument.

Capillary zone electrophoresis is not only the simplest form of capillary electrophoresis, but also the most common one. General schematic of a capillary zone electrophoresis instrument is presented in Figure 2.7. The instrument includes a high voltage power supply (0-60kV), a polyimide-coated capillary (internal diameter < 200 μm), two buffer reservoirs to hold both the capillary and the electrodes which are connected to the power supply, and a detector (Integrator or Computer). During the separation process, an appropriate separation buffer which is at the desired pH is filled into the capillary from the inlet. Both the ends of capillary and the electrodes are placed into the buffer

reservoirs, and immersed in the buffer. Up to 30,000V is applied to the system. The particles in the sample migrate with an electrophoretic mobility determined by their charge and mass, and finally pass a detector where information is collected and stored by an integrator or computer [17].

2.1.2.6. Magnetic activated cell sorting

Magnetic particles now are widely used in isolation of bacteria [18-22], viruses [23-28], and other cells [29-32]. The magnetic bead cell sorting procedure is illustrated in Figure 2.8.

Figure 2.8 Conceptual illustration of magnetic bead cell sorting procedure [33]. (a) Injection of magnetic beads; (b) Separation and holding of beads; (c) Flowing samples; (d) Immobilization of target antigen; (e) Release to biosensor or bioreactor.

Antibody-coated beads are introduced on the electromagnet (Figure 2.8(a)) and are separated by applying magnetic fields. While the antibody coated beads

attach on the channel wall by the magnetic force (Figure 2.8(b)), antigens are injected into the channel (Figure 2.8(c)). Only target antigens are immobilized, and then separated onto the magnetic bead surface due to antibody/antigen interaction (Figure 2.8(d)). Other antigens get washed out with the flow. In a subsequent optional step, labeled secondary antibodies can be introduced and incubated with the immobilized antigens. After that, the chamber is rinsed to remove all unbound secondary antibodies. Finally, the magnetic beads are released to a sensing chamber of the bio-assay (Figure 2.8(e)), and the bio-separator is ready for the next sample.

The first magnetic particles, agarose-polyacrylamide beads, were reported by Guesdon and Avrameas [34]. A magnetic rack was used to separate the magnetic polyacrylamide-agarose beads from the liquid phase, from then on, a new way which is not like the time-consuming multiple centrifugations appears, and develops fast.

Lund et al. [35] coated magnetic beads with monoclonal antibodies which could react with the K88 (F4) fimbrial antigen responsible for colonization of piglet intestines. Skjerve and Olsvik [36] detected as low as 16 cells/ml of *Salmonellas* from a mixed sample containing *Salmonellas* and *E. coli* by using a commercial immuno-purified polyclonal goat IgG against *Salmonella* structural antigen, which is linked covalently to magnetic particles. In the mixture, the concentration of *E. coli* is 1000 times more than *Salmonellas*, which led to their detection without pre-enrichment. In 2001, by using mono-disperse magnetic

beads in concentrating bacteria in suspension, Jonathan Porter and Roger W. Pickup [37] developed a gram staining technique in which heat fixation of organisms, electrical power, or a microscope are not required. What's more, the gram-negative and gram-positive bacteria were identified macroscopically based on the color.

Nilgün Başar and his coworkers [38] used dye-attached mPHEMA beads to purify lysozyme. The purity of the desorbed lysozyme was about 87.4% with recovery about 79.6%.

With the advanced development of magnetic beads, now magnetic particles are one of the main cell-sorting methods. Table 2.4 lists the commercially available magnetic particles [39].

Table 2.4: Summary of commercially available magnetic particles [39].

Name	Diameter (μm)	Composition	Immobilized compounds	Manufacturer
Isolex super-magnetic beads	4.5	Polystyrene	Secondary Abs	Baxter Healthcare
Dynabeads M-280	2.8	Polystyrene	Secondary Abs,	Invitrogen Dynal AS
Dynabeads M-450	4.5		Anti-CD Abs,	
Dynabeads M-500	5		Streptavidin	
Magnetic particles	1	Polystyrene	Streptavidin	Boehringer
Magnetic microparticles	1–2	Polystyrene	Protein A	Polysciences
MagaBeads	3.2	Polystyrene	Secondary Abs, streptavidin, protein A, protein G	Cortex Biochem.
SPHERO	1–4.5	Polystyrene	Secondary Abs,	Spherotech

magnetic particles			streptavidin, Biotin	
XM200 Microsphere	3.5	Polystyrene	Secondary Abs, protein A	Advanced Biotechnologies
MACS microbeads	0.05	Dextran	Secondary Abs, anti-CD Abs, streptavidin, biotin, anti-FITC, Anti-PE	Miltenyi Biotec
Magnetic Nanoparticles	0.09-0.6	Starch, dextran, Chitosan	Streptavidin, protein A, Biotin	Micro-caps
Ferrofluids	0.135 , 0.175	Modified hydrophilic Protein	Secondary Abs, streptavidin, protein A	Immunicon

2.1.2.7. Summary

Most of the reported concentration methods process a limited initial sample volume, and confines the target in a limited final volume. Additionally, these concentration methods suffer from low-yield, purity or throughput. Centrifugation and its variant, density gradient centrifugation are well-established techniques, and they are widely used as a concentration method, because they are simple to perform but to process the large volume samples they require multiple operations batch wise which makes it tedious and laborious and time consuming method. There are reports of filter-based methods [40] for concentration of cells, but their clogging and slow assay time offers are limitations to this technique. To circumvent these issues, solid phase extraction methods have been developed. These include usage of magnetic particles, silica beads and gels. A disadvantage of packed beads method is that the free beads which are not strongly linked to the channels have chances of accidental exhaustion with the flow of solution. Moreover beads are quite expensive; it will be impractical to use this method in the sample with large volumes. Although fluorescence activated cell sorting (FACS) shows high performance in cell recovery and purity [41], the serial screening of each cell limits the throughput, typically in the range between 10^4 and 10^5 cells/second [42]. In contrast, with respect to throughput, magnetic activated cell sorting (MACS) is capable of concentrating large number of target cells in parallel [43]. However, the purity and yield in MACS have large variances [44]. Another method gaining popularity for concentration of cells and their sorting is dielectrophoresis. Eric T. Lagally [45] demonstrated cell concentration and

genetic detection using DEP in an integrated micro-system. The limitation offered by DEP is in terms of the time it takes to isolate or concentrate cells since the flow rate, which is usually used in $\mu\text{l/hr}$ resulting in very long assay time. Table 2.5 summarizes the limitations of various concentration methods.

Table 2.5: Limitations of the commonly used cell concentration methods.

Method	Limitations
Centrifugation/density gradient centrifugation	Low-yield Mostly batch process. Requires multiple steps for large volumes of samples
Fluorescence activated cell sorting (FACS)	Tedious Low-throughput Requires tagging of cells with fluorescence.
Magnetic activated cell sorting (MACS)	Tedious Low-purity Requires antibody tagged magnetic beads
Filter-based sorting	Clogging Fouling Frequent replacement for continuous processing methods

Dielectrophoresis (DEP)	<p>Many parameters for optimization</p> <p>Long analysis time due to slow flow rate.</p> <p>Optimized parameters are applicable only to system which is studied. Cannot be generalized.</p> <p>Specific requirements like conductivity, purity of sample.</p>
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2.2. Bacterial adhesion

2.2.1. Introduction to bacterial adhesion

After cell concentration, most of the cells stuck on a surface. Many bacteria have been shown to exist predominantly adhered to surfaces in contact with liquids [46]. It is critical to suppress bacterial adhesion to the surface so that the concentrated cells can be easily transported into the downstream analytical systems.

2.2.2. The dominating factors for bacteria adhesion to a surface

Lots of factors can influence the bacterial adhesion, such as surface charge, hydrophobicity, surface roughness and surface micro-topography [47].

2.2.2.1. Surface charge

In some instances, surface charges can determine the bacterial adhesion on a substratum. Most bacterial surfaces are negatively charged at physiological pH [48]. Jucker et al [49] reported that compared with *Pseudomonas putida* with a negative net charge at physiological pH, *S.maltophilia* with a positive net charge had a higher attachment efficiency to Glass and Teflon(both of them have a negative surface charge). However, Gilbert et al. [50] noted that the adhesion of the *E. coli* cells on the surface can be reduced by increasing negative charge on the *E. coli* surface, but it could not work on *S. aureus*, which demonstrated that

surface charge may be one of the dominating factors to bacterial adhesion, but it was not the only reason.

2.2.2.2. Hydrophobicity

It was widely accepted that hydrophobic interactions were responsible for the cell attachment to surfaces [51]. Freitas, A.M et al [52] reported that substrates with a higher hydrophobicity could lead to a lower critical hydrodynamic force required to detach the particles in simple shear flow. By modifying hydrophobicity on one polymeric surface, and measuring the adhesion of *Staphylococcus epidermidis*, Haiying Tang et al [53] demonstrated that bacterial adhesion decreased when hydrophobicity was increased.

2.2.2.3. Surface roughness and surface micro-topography

Surface roughness and surface micro-topography also play an important role in bacterial adhesion [54]. By testing the attachment of three strains of bacteria on the silicon groove with various depths, Scheuerman et al. [54] demonstrated that the effect of the substratum topography on bacterial adhesion was significant. For this case, the greatest attachment was achieved on the downstream edges of the grooves. Tang, H. [53] reported that surface roughness had a significant effect on bacterial adhesion and colonization only when the root-mean-square roughness was higher than 200 nm. Katainen, J. [55] investigated the effect of roughness on bacterial adhesion in another direction. He looked at adhesion as an interplay between particle size and surface roughness and commented that adhesion of particles smaller than or similar in size to the asperities depend

mainly on the size and shape of the asperities and only weakly on the size of the particle. For large particles also the particle size has a significant effect on the adhesion.

Chapter 3 Methodology

3.1. Background

The methods for sample concentration have already been introduced in Chapter 2. Each method has their advantages and disadvantages. Current centrifugation has the disadvantages of low-yield, mostly batch process, and multiple steps for large volumes of samples. If these disadvantages of the centrifugation are minimized, centrifugation will be an ideal method in sample concentration.

The reasons for the low-yield and tedious of the centrifugation are investigated. Conventional centrifugations usually take the steps as following:

- 1) Load the rotor (Figure 3.1) with sample,
- 2) Accelerate to operating speed,
- 3) Run for a specific period of time,
- 4) Decelerate to a stop,
- 5) Unload the sample.

These steps must be repeated before processing the entire sample.

If the sample processed is in large quantity, low sedimentation rate, long acceleration/deceleration time, and small capacity, the conventional way will be time consuming [8].

There is another method named continuous flow centrifugation, where large volumes can be significantly reduced with centrifugation at high speed without

the tedium of filling and decanting a lot of centrifuge tubes, or frequently starting and stopping the rotor. This combination of high centrifugal force and high throughput makes continuous flow processing particularly useful for the large-scale collection of viruses (either for research purposes or for the preparation of commercial vaccines), the sedimentation of bacteria, and the pelleting of sub-cellular fractions.

Two predominant types of continuous centrifuges are disks and decanters [56, 57]. They cover a wide range of applications with flow rates from less than 1 to more than 4000L/min, slurry particle sizes from 0.1 μ m to 25mm, solids concentrations of less than 0.2% to 70+% w/w, and Gs from 500 to over 15000g. The disk centrifuge handles the small end of the size spectrum from 0.1 to 50 μ m particles by using high G in the range of 4000-15000g. On the other hand, the decanter (Figure 3.1) handles the coarser end of the particle sizes from 1 μ m to 10mm using moderate to high G in the range of 500-10000g. Also, the disk is typically used for both dilute as well as concentrated slurry [8].

Figure 3.1 Cross section of a continuous flow rotor [6].

Thus, continuous flow centrifugation can be quite useful to concentrate samples from a large volume. However, there are limitations with current continuous centrifugation methods. The concentrated particles cannot be easily collected and transported into other functional modules, such as a cell sorter and a PCR system. Table 3.1 lists the multiple fluidic components in the centrifugal chip based microfluidic platforms, but still there is no report on the usage of particle concentration component in the centrifugal chip based microfluidic platform. The aim of this research is to design a chip that can be used in continuous flow centrifugation with integrated particle collection and integrated particle transport to downstream functional modules (Figure 3.2). Two methods of integrated continuous flow centrifugation, the Point Capture method and the Distributed Surface Capture method, are used in chip design.

Polydimethylsiloxane (PDMS) coated channel was introduced to modify the roughness of the channel wall so that the bacterial adhesion to the channel during cell collection could be suppressed.

Table 3.1: Summary of fluidic components in the centrifugal chip based microfluidic platforms.

Functions	Mechanisms	Reference
Propulsion	Fluid propulsion is achieved through rotationally-induced hydrostatic pressure and depends on rotation rate, geometry and location of channels, and fluid properties.	[58][59] [60][61] [62][63] [64]
Valving	Fluid gating is accomplished by using capillary forces or hydrophobic forces. Flow is impeded until rotationally induced pressure overcomes the capillary pressure.	[58][59] [61][62] [63][65]
Mixing	Turbulence can be created on the centrifugal chip by emptying two narrow streams to be mixed into a common chamber. The streams violently splash against a common chamber wall so that they can be effectively mixed.	[58][59] [64][65] [66][67] [68]
Volume definition and	Reagents are introduced by the capillary force exerted by hydrophilic surfaces into the common channel and defined volume chambers until a hydrophobic valve stops the flow.	[58][64] [69]

common distribution channel	<p>When all of the defined volume chambers are filled, the centrifugal chip is spun at a velocity large enough to move the excess liquid from the common channel into the waste.</p> <p>Although there is sufficient centripetal force to empty the common channel, the velocity is not so high as to allow liquid to move past the hydrophobic valve and the well-defined volume chambers remain filled. These precisely defined volumes can be introduced into the subsequent fluidic structures by increasing the centrifugal chip angular momentum until the centrifugal force allows the liquid to move past the hydrophobic barriers</p>	
Packed columns	<p>A reverse phase chromatography column material is packed in a microfluidic channel and protein is adsorbed on the column from an aqueous sample as it passes through the column under centrifugally controlled flow rates. A rinse solution is subsequently passed through the column, and finally an elution buffer flows through to remove the protein and carry it into the fluidic system for further processing.</p>	[58][69]

Figure 3.2 Continuous flow centrifugation with integrated particle collection and integrated particle transport to downstream functional module.

The working principle of the Point Capture method will be discussed in Section 3.4.1.2, and the working principle of the Distributed Surface Capture method will be discussed in Section 3.4.2.2. The integration of the sample concentration, particle collection and particle transport to downstream functional modules will be demonstrated in Section 3.4.3.

3.2. Basic definitions and mass balance equations

A single-stage continuous flow concentration system can be schematically drawn as in Figure 3.3:

Figure 3.3 Schematics of a single-stage continuous flow concentration system, M is the mass of the feed (in kg), M_c is the mass of the particles concentrated in the J channel pattern chip (in kg), M_s is the mass of the particles in the supernatant (in kg).

The total mass of the feed must be equal to the sum of the total masses of the products, i. e.

$$M = M_c + M_s. \quad (3.1)$$

If the total concentrating efficiency E_T is now defined as simply the ratio of the mass of all particles separated (M_c) to the mass of all solids fed into the J channel pattern chip (M), i.e.

$$E_T = M_c/M = 1 - M_s/M. \quad (3.2)$$

This equation can be used to calculate the concentrating efficiency of both fluorescent beads concentration and *E.coli* cells concentration.

3.3. Outline of experiments

For concentrating, collecting and transporting particles, three groups of experiments were carried out.

1) Methods for concentrating, collecting and transporting fluorescent beads (Section 3.4)

Fluorescent beads were used as the mimic of the bacteria for particle concentration, collection and transport in our proposed chips. This section introduced 3 chips to continuously concentrate, collect and transport fluorescent beads. The chips included the point capture chip, the distributed surface capture chip, and the integrated particle transport chip. In this case, the point capture chip which is a J-channel chip was used to continuously concentrate and collect fluorescent beads. The distributed surface capture chip which is a spiral channel chip also was used to continuously concentrate and collect fluorescent beads. The integrated particle transport chip was used to transport the fluorescent beads into downstream functional modules.

2) Methods for concentrating, collecting *E.coli* cells (Section 3.5)

After the experiments were carried out in last section, it was demonstrated that fluorescent beads could be continuously concentrated, collected and transported by our proposed chips. It was also very important if the continuous concentration and collection of the real environmental sample could be realized by our chips. Thus, in this section, the *E.coli* cells, one of the most important

environmental pathogens, were chosen as the sample to be concentrated and collected.

3) Methods for surface treatment of poly(methyl methacrylate) (PMMA) channels (Section 3.6)

It was demonstrated that both the fluorescent beads and the *E.coli* cells could be concentrated and collected by our proposed chips. However, although the particle concentrating efficiency could reach a high level, the droplet based particle collecting efficiency were still need to be promoted. Several papers and patents reported that poly (ethylene oxide) (PEO) based polymers could be deposited on the surfaces to effectively inhibit the cell adhesion [70-74]. We treated PMMA sheets with PluronicTM F108 (BASF, Germany) solutions in the different concentrations to investigate the relationship between Pluronic F108 concentration and cell detachment.

3.4. Methods for concentration, collection and transport of fluorescent beads

In this section, three chips were introduced to continuously concentrate, collect and transport fluorescent beads. The methodology of each chip was discussed in the following.

3.4.1. Methodology for chip design 1 (The point capture chip)

The Point Capture method is to concentrate particles in a small zone/point. In this section, the Point Capture chip, including the design and fabrication of the chip, the working principle of the Point Capture method, instruments setup, characterization, the experimental procedure of the Point Capture method and the results will be discussed.

3.4.1.1. The design of the Point Capture chip

The design of the point capture chip employed a spiral channel pattern made of four poly(methyl methacrylate) (PMMA) layers (Figure 3.4). The first layer was a substrate which was used to enclose the channel, and the second layer was the channel layer in which the J channel and waste chamber was provided. The J channel was the main functional component in particle concentration, and the waste chamber was the place where supernatant was collected during particle concentration. The concentration zone/point which was at the bottom of the J-channel was the place where particles were concentrated. The third layer was used to increase the volume of the waste chamber. Finally, the fourth layer was used to provide the air vents and sample loading port. The positioning holes of each layer were used to align the layers during thermal bonding.

(a)

(b)

Figure 3.4 Schematics of the J-channel pattern chip structure. (a) 3D structure of the point capture chip; (b) structure of Layer 2.

3.4.1.2. Working principle of the Point Capture method

A J-channel pattern chip is used to demonstrate the working principle of the Point Capture method. First of all, the particle suspension is loaded from sample loading port into the empty J-channel pattern chip (Figure 3.5(a), (b)). Then, the chip begins to rotate, and the centrifugal force of the particles concentrates the particles into concentration zone/point (Figure 3.5(c)). After all the sample suspension has flowed through the Point Capture chip, the rotation of the rotor is stopped and air is blown into the chip from the sample loading port to extract the supernatant in the channel out (Figure 3.5(d)-(g)). Then, a small droplet with the collected particles is formed in the channel, and air is blown into supernatant removal port to roll back the droplet (Figure 3.5(h)). By air pumping, the droplet with the collected particles is kept moving around the concentration zone/point to collect the rest of the concentrated particles into the droplet (Figure 3.5(h)-(j)).

Figure 3.5 Working principle of the Point Capture method. (a) place an empty J channel chip; (b) load the sample into the chip; (c) Spin the chip to concentrate particles; (d-g) pump air into the channel to extract the supernatant, and get a droplet; (h-j) pump air to move the droplet forward and back to collect the rest particles.

3.4.1.3. The fabrication of the Point Capture chip

The chip fabrication process consists of several important steps. Graphical illustrations of the chip components were drawn using Corel Draw vector graphics editor. Then, poly(methyl methacrylate) (PMMA) sheets were cut into the graphical patterns by laser cutter. After that, the four layers were bonded together by the vulcanizer. Finally, polydimethylsiloxane (PDMS) was injected into the channel to form a thin PDMS film on the channel wall to decrease the roughness of the channel wall. The specific fabrication process was as follows:

1) Structure drafting by Corel Draw

The model is created on the workspace in the middle rectangular zone (Figure 3.6). The tool box which is on the left side contains the sketching tools such as select tool, fill tool, etc. The attributes bar which is on the top of the working interface is the place to set the scope of the workspace and the units. The column on the right side is the object properties bar, where the properties of the objects such as the line color, line width, and line style can be modified.

Figure 3.6 The graphic interface of Corel Draw vector graphics editor.

The illustrations of the four layers in Corel Draw were shown in Figure 3.7. The parameters of the laser setting for 1.5 mm thick PMMA sheets were Power 100%, Speed 8%, PPI 1000 and the parameters of the laser setting for 1 mm thick PMMA sheets were Power 40%, Speed 8%, PPI 1000.

Figure 3.7 Layer pattern of J-channel pattern chip (unit: mm). (a) Layer 1: 1mm thick; (b) Layer 2: 1.5mm thick; (c) Layer 3: 1.5mm thick; (d) Layer 4: 1mm thick.

2) Laser cut by VSL 2.30 laser cutter

After the structure was drafted in Corel Draw vector graphics editor, the structure was transported into VSL 2.30 print preview (Figure 3.8). After the 'settings' button was clicked, a new window to configure the parameters of laser cutting will pop up (Figure 3.9). Power, speed, PPI (points per inch) can be configured to control the cutting quality. Power refers to the power of the laser source, and the speed refers to the speed of the rotor where laser source is positioned. When the laser machine cuts a curve in material such as PMMA, it doesn't cut the curve directly. In fact, the laser cutter cuts the curve point by

point. The minimum distance between two points, determines the accuracy of the curve. PPI represents the largest number of points that can be cut per inch. Larger PPI leads to a smaller distance between two points so that the curve will be more smoothly and accurately.

Figure 3.8 The print preview of VLS 2.30 laser cutter.

Figure 3.9 The parameter settings of VLS 2.30 laser cutter. Power: the power of laser. Source; Speed: the speed of the rotor where laser source is positioned; PPI: point per inch.

3) Thermal bonding by the vulcanizer

The patterns of four layers were generated in PMMA sheets. Then, 127°C thermal bonding for 30 minutes was used to bond the PMMA sheets together.

The photo of the fabricated J-channel pattern chip was shown in Figure 3.10

Figure 3.10 Photo of fabricated J-channel pattern chip for concentrating fluorescent beads.

4) Polydimethylsiloxane (PDMS) deposition in the channel

The pre-polymer (Sylgard 184-A) and curing agent (Sylgard 184-B) were mixed on a dish at a weight ratio of 10:1. In order to degas the mixed PDMS, the dish was placed in a vacuum chamber for 30 min. The mixture was injected into the channel of the fabricated chip, and a high pressure air gun was used to keep pumping air into the channel so that only a thin PDMS film would be deposited on the channel wall. Finally, the chip was baked in an oven at 70–80 °C for 1 h.

3.4.1.4. Materials

Yellow Green fluorescent microspheres with a 1 μm diameter (Product No. SKU#F-8823, Invitrogen Corp. Eugene, Oregon, CA, USA specified absorption

wavelength 505 nm, specified emission wavelength 515 nm) were diluted 1:500 in pure water, and placed into a 15 ml plastic tube as the sample suspension.

A Sylgard 184 kit (Dow Corning Corporation, Midland, MI) containing Polydimethylsiloxane (PDMS) pre-polymer (Sylgard 184-A) and curing agent (Sylgard 184-B) was employed to PDMS deposition.

3.4.1.5. Instruments setup

The schematic drawing of centrifugal system is shown in Figure 3.11.

Figure 3.11 Schematic drawing of the centrifugal system for particle concentration.

The Point Capture chip was placed on rotating plate, and then the air in the vacuum point was pumped out by the vacuum pump so that vacuum would be generated in the vacuum point. After that, the atmospheric pressure created during the process held the chip on the rotating plate tightly. The controller of the rotating machine can be programmed to control rotating speed, and rotating time. Syringe pump pumps the sample suspension from the dispensing nozzle into the sample loading port.

3.4.1.6. Characterization of the concentration of fluorescent particles in the J channel chip

As described in Section 3.2, the total concentrating efficiency E_T is defined as the ratio of the mass of all particles separated (M_c) to the mass of all solids fed into the J channel pattern chip (M). From Equation (3.2) $E_T = M_c/M = 1 - M_s/M$, we can get:

$$E_T = 1 - M_s/M = 1 - Q_s C_s / QC = 1 - (Q - Q_c) C_s / QC. \quad (3.3)$$

Since compared with Q , Q_c can be neglected, the total concentrating efficiency E_T can be calculated by:

$$E_T = 1 - (Q - Q_c) C_s / QC = 1 - QC_s / QC = 1 - C_s / C, \quad (3.4)$$

where:

M is the mass of the feed (in kg)

M_c is the mass of the particles concentrated in the J channel pattern chip (in kg)

M_s is the mass of the particles in the supernatant (in kg).

Q is the volume of the feed (in mL)

Q_c is the volume of the fluid in the J channel pattern chip (in mL)

Q_s is the volume of the supernatant (in mL)

C is the average concentration of the particles in the feed

C_c is the average concentration of the concentrated particles in the in-chip fluid.

C_s is the average concentration of the particles in the supernatant.

To the suspension of fluorescent particles, the fluorescent strength (FS) can represent the concentration of fluorescent particles (C), thus E_T can be obtained by:

$$E_T = 1 - C_s/C = 1 - FS_s/FS. \quad (3.5)$$

A TECAN Genios Multifunction Fluorescence, Absorbance and Luminescence Microplate Reader equipped with UV-VIS Fluorescence and Absorbance Reader for top- and bottom- read assays was used for all fluorescence and bright-field measurements.

3.4.1.7. Instrumentations

A TECAN Genios Multifunction Fluorescence, Absorbance and Luminescence Microplate Reader (Figure 3.12) equipped with UV-VIS Fluorescence and Absorbance Reader for top- and bottom- read assays was used for all

fluorescence and bright-field measurements. A 485 nm filter was chosen as the excitation filter and a 535 nm filter was chosen as the emission filter.

Figure 3.12 Photo of a TECAN GENios Multifunction Fluorescence, Absorbance and Luminescence Microplate Reader for the measurements of fluorescence.

A Spin Processor (Figure 3.13) with vacuum chucks_1.75” and 4” (Figure 3.14) (Product No. WS-650S-6NPP/LITE, Laurell Technologies Corp., North Wales, PA, USA) was used for centrifugation.

Figure 3.13 Photo of the spin processor for spinning chips.

Figure 3.14 Photo of the vacuum chucks of the spin processor for chips holding.

(a) 4" vacuum chuck; (b) 1.75" vacuum chuck.

A syringe pump (Figure 3.15) (Product No. NE-1000, New Era Pump Systems, Inc., Wantagh, NY, USA) was used for controllable sample pumping.

Figure 3.15 Photo of a programmable syringe pump.

A vulcanizer (Product No. P30, Kerr Corp., Romulus, MI, USA) was used for thermal bonding (Figure 3.16).

Figure 3.16 Photo of a temperature controllable vulcanizer for thermal bonding.

A laser cutter (Figure 3.17) (Product No. VLS 2.30, Universal Laser Systems Inc., Houston, TX, USA) was used to cut the layer structures which were drawn by Corel Draw vector graphics editor into PMMA sheets.

Figure 3.17 Photo of VLS 2.30 laser cutter.

A fluorescence imaging system is used to observe fluorescence labeled particles (Figure 3.18). The optics of the instrument was designed to measure the fluorescence of SYBR Green I/EvaGreen, DNA intercalating dye. The EvaGreen fluorophore was excited using an array of blue LED (Marl International Ltd, Cumbria, UK) centered at 480 nm and fixed at an angle of 45° to the plane of the Point Capture chip surface to prevent interference of the excitation light on the light path of the detection unit. The excitation light from the blue LED array was filtered using a band-pass filter (465-495nm, Chroma Technologies Corp, Brattleboro, USA), and the emission light from the micro-reactor was filtered using another band-pass filter (515-555 nm, Chroma

Technologies Corp, Brattleboro, USA) before being detected by a CCD camera (DTA, Pisa, Italy).

Figure 3.18 Fluorescence imaging system for the observation of particle concentration and collection: (a) Framework of fluorescence imaging system; (b) Photo of fluorescence imaging system.

3.4.1.8. Experimental procedure

The experimental procedure for the Point Capture chip is shown in the following:

Experiment for particle concentration:

- 1) Prepare the sample suspension by diluting 100 μ l micro-bead suspension into 15ml water.
- 2) Seal the vent 2 by a silicon block, and fasten it with transparent scotch tape.
- 3) Place the chip on the spin coater.
- 4) Shake the sample suspension to make a uniform concentration.

- 5) Extract 10ml sample suspension into the syringe, and put the syringe on the syringe pump.
- 6) Choose the sample pumping speed.
- 7) Pump 200 μ l first to wet the tube so that the particle loss in the transport process will be decreased.
- 8) Pump 250 μ l as the control.
- 9) Open the spin coater, and start the program.
- 10) After the spin coater reaches to the target speed, begin to pump 500ul sample into the chip.
- 11) After pumping 500ul sample, stop the spin coater.
- 12) Take out the chip from the spin coater.
- 13) Remove the scotch tape, and take out the silicon block.
- 14) Extract 200 μ l supernatant from the supernatant chamber.
- 15) Shake the control sample.
- 16) Get 100 μ l control sample, and put into one well of the 96-spot plate (For example: Well B2).
- 17) Shake the supernatant.
- 18) Get 100 μ l supernatant, and put into another well of the 96-spot plate. (For example: Well B3).
- 19) Put 100 μ l deionized water into another well of the 96-spot plate. (For example: Well B4).
- 20) Put the 96-spot plate into the TECAN Genios Multifunction Fluorescence, Absorbance and Luminescence Microplate Reader to test the fluorescent strength.

- 21) Wash the chip and start a new experiment.

Experiment for particle collection:

- 1) After step 14 in the experiment of particle concentration, seal the vent 1 with transparent scotch tape.
- 2) Place a 10ml syringe onto the syringe pump.
- 3) Link the syringe to tubing, and connect the other side of the tubing with vent 2.
- 4) Program the syringe pump to set the pumping speed and pumping volume.
- 5) Start the syringe pump to move the droplet passing through the concentration zone to collect concentrated particles. Take photos of the droplet movement by fluorescent imaging system.
- 6) Open the “withdraw” button to move in the opposite direction.
- 7) Repeat step 5 and step 6 for several times.
- 8) Save all the photos and wash the chip.

After the experiment, fluorescent beads suspensions were concentrated in different feed rates at the rotating speed of 5000RPM and 4000RPM, respectively. Generally speaking, the concentrating efficiency increases when feed rates decrease, and the larger rotating speed can lead to a larger concentrating efficiency. The feed rates only can reach 10 ml/h at the concentrating efficiency of 80% with the rotating speed of 5000 rpm. The

photos of particle collection showed that the concentrated particles could be collected into the droplet. The details of the results were discussed in Chapter 4.

3.4.2. Methodology for chip design 2 (The Distributed Surface Capture chip)

The Point Capture method can only concentrate particles in one small zone/point so that the feeding flow rate is quite small (around 10mL/h). Thus, the Distributed Surface Capture chip which has a much longer concentration zone (along the channel) is developed to increase the feeding flow rate.

3.4.2.1. The design of the Distributed Surface Capture chip

The design of the distributed surface capture chip employed a spiral channel pattern made of five layers (Figure 3.19 (a)). The first layer (1mm) was a substrate which was used to enclose the channel, and the second layer (3mm) was the channel layer in which the spiral channel was provided. The spiral channel was the main functional component in particle concentration. The third layer (1mm) enclosed the channel, and provided a sample loading port and a supernatant removal port. The fourth layer and the fifth layer were introduced to prevent sample suspension from spilling out during centrifugation. The fourth layer (5mm) which was thicker than other layers was used to generate a buffer chamber to decrease the momentum which can spill the sample out of the chip; while the fifth layer (1mm) was used as a baffle to decrease the open area where the sample may spill out.

Figure 3.19 Schematics of the spiral chip structure and spiral channel pattern.

(a) 3D schematics of the spiral chip structure; (b) the structure of Layer 2.

3.4.2.2. Working principle of the Distributed Surface Capture chip

A spiral channel pattern chip is used to demonstrate the working principle of the Point Capture method. The particle suspension is loaded from the sample loading port into the empty spiral channel pattern chip (Figure 3.20 (a), (b)). Then, the chip begins to rotate. As a result of the centrifugal force and adhesive force between the particles and the channel wall, the particles will be concentrated along the channel wall (Figure 3.20 (c), (d)). The longer the channel is, the lesser particles are lost in the supernatant. After all the sample suspension has flowed through the Distributed Surface Capture chip, we keep rotating until all the water in the channel is thrown out (Figure 3.20 (e), (f)).

Then, a DI-water droplet is blown into the chip from the sample loading port to collect the concentrated particles (Figure 3.20 (g)). The DI-water droplet moves in the channel until most of the concentrated particles are collected into the droplet (Figure 3.20 (h)-(j)).

Figure 3.20 Working principle of the Distributed Surface Capture method. (a) place an empty spiral chip; (b) load sample into the chip; (c-d) spin the chip to concentrate particles; (e-f) stop the spinning and extract the supernatant away; (g-j) load a water droplet to collect particles.

3.4.2.3. The fabrication of the Point Capture chip

The fabrication process of the distributed surface capture chip was the same as the point capture chip (Section 3.4.1.2). The illustrations of the five layers in Corel Draw were shown in Figure 3.21. The parameters of the laser setting for 3 mm thick PMMA sheets were Power 90%, Speed 4%, PPI 500, for 5 mm thick PMMA sheets were Power 100%, Speed 2%, PPI 500 and for 1 mm thick PMMA sheets were Power 40%, Speed 8%, PPI 1000.

Figure 3.21 Layer pattern of spiral pattern chip (unit: mm). (a) Layer 1: 1 mm thick; (b) Layer 2: 3 mm thick; (c) Layer 3: 1mm thick; (d) Layer 4: 5 mm thick; (e) Layer 5: 1 mm thick.

3.4.2.4. Materials

The material in this experiment is the same as the one in Section 3.4.1.

3.4.2.5. Instruments setup

The schematic drawing of centrifugal system is shown in Figure 3.22.

Figure 3.22 Schematic drawing of the centrifugal system for particle concentration.

The spiral chip was placed on rotating plate, and then the air in the vacuum point was pumped out by the vacuum pump so that vacuum would be generated in vacuum point. After that, the atmospheric pressure held the chip on the rotating plate tightly. The controller of the rotating machine could be programmed to control rotating speed, and rotating time. Sample loading pump pumps sample suspension from the dispensing nozzle into the sample loading port. The sample loading pump is powered by a DC power supply (0-12V).

3.4.2.6. Characterization of concentrating fluorescent beads in the spiral chip

The characterization in this experiment is the same as the one in Section 3.4.1.6.

3.4.2.7. Experimental procedure

The experimental procedure of the Distributed Surface Capture method is shown in the following:

Experiment for particle concentration:

- 1) Prepare the sample suspension by diluting 1ml micro bead suspension into 200ml water.).
- 2) Place the Distributed Surface Capture chip on the rotating plate.
- 3) Press the button of “Vacuum” to hold the chip on the rotating plate.
- 4) Set rotating parameters such as the rotating time and speed by programming in the controller of the rotating machine.
- 5) Close the cover of the rotating machine, and begin to rotate the rotating plate.
- 6) Insert the dispensing nozzle into the sample loading port of the chip, and start to load the sample suspension by turning on the power supply of the sample loading pump.
- 7) After the entire sample suspension flow through the chip, keep rotating until all the water is thrown out.
- 8) Collect the supernatant that has flowed through the chip.
- 9) Extract 200 μ l supernatant from the supernatant chamber.
- 10) Shake the control sample.
- 11) Get 100 μ l control sample, and put into one well of the 96-spot plate (For example: Well B2).
- 12) Shake the supernatant.

- 13) Get 100 μ l supernatant, and put into another well of the 96-spot plate.
(For example: Well B3).
- 14) Put 100 μ l deionized water into another well of the 96-spot plate. (For example: Well B4).
- 15) Put the 96-spot plate into the TECAN Genios Multifunction Fluorescence, Absorbance and Luminescence Microplate Reader to test the fluorescent strength.
- 16) Wash the chip and start a new experiment.

Experiment for particle collection:

- 1) After step 7 in the experiment of particle concentration, place a 10ml syringe onto the syringe pump.
- 2) Link the syringe to tubing, and connect the other side of the tubing with the outlet of the chip.
- 3) Program the syringe pump to set the pumping speed and pumping volume.
- 4) Inject one DI-water droplet into the chip.
- 5) Start the syringe pump to move the droplet passing through the concentration zone to collect concentrated particles. Take photos of the droplet movement by fluorescent imaging system.
- 6) Open the “withdraw” button to move in the opposite direction.
- 7) Repeat step 5 and step 6 for several times.
- 8) Save all the photos and wash the chip.

After the experiment, fluorescent beads suspensions were concentrated in different feed rates at the rotating speed of 5000RPM. Generally speaking, the concentrating efficiency increases when feed rates decrease, and the larger rotating speed can lead to a larger concentrating efficiency. The feed rate can reach up to 120 ml/h at the concentrating efficiency of 80% with the rotating speed of 5000 rpm. The details of the results were discussed in Chapter 4.

3.4.3. Methodology for integrated particle transport (Integrating the Point Capture method with genetic testing capability)

Section 3.4.1 and Section 3.4.2 demonstrated that the particles can be concentrated by the Point Capture method or the Distributed Surface Capture method, and collected into a droplet. This section is to demonstrate that the droplets collected in the Point Capture chip or the Distributed Surface Capture chip can be easily transported into downstream functional modules, such as the array of micro-reactors for genetic analysis.

3.4.3.1. Integrated particle transport chip design

The design of the integrated particle transport chip employed a spiral channel pattern for the Point Capture method, an array of micro-reactors, and a waste chamber made of three layers (Figure 3.23). The first layer was used to enclose the channel. The second layer was the channel layer, including the J-pattern channel, the array of micro-reactors channel, and the waste chamber. The third layer had the air vents.

(a)

(b)

Figure 3.23 Schematics of the integrated particle transport chip. (a) 3D structure of the integrated particle transport chip; (b) structure of Layer 2.

3.4.3.2. The fabrication of the Point Capture chip

The fabrication process of the integrated particle transport chip was the same as the point capture chip (Section 3.4.1.3). The illustrations of the three layers in Corel Draw were shown in Figure 3.24. The parameters of the laser setting for 3 mm thick PMMA sheets were Power 90%, Speed 4%, PPI 500, and for 1 mm thick PMMA sheets were Power 40%, Speed 8%, PPI 1000. The photo of the fabricated J-channel pattern chip was shown in Figure 3.25.

Figure 3.24 Layer pattern of integrated particle transport chip.(a) Layer 1: 1.5mm thick; (b) Layer 2: 3 mm thick; (c) Layer 3: 1.5 mm.

Figure 3.25 Photo of the fabricated particle transport chip.

3.4.3.3. Working principle of the integrated particle transport chip

When air vents above the array of micro-reactors and absorbent port are sealed, the integrated particle transport chip becomes a Point Capture chip. As illustrated in Section 3.4.1, the particles can be concentrated in the concentration zone/point, and be collected into a DI-water droplet. Then, after sealing the waste port, and opening air vents above the array of micro-reactors and absorbent port, droplet can be moved into the array of reactors for high-throughput genetic analysis. The excess portion of droplet after the filling of the micro-reactor array is absorbed by the absorbent at the end of the sample loading channel.

3.4.3.4. Experimental procedure

Since the experiments of the particle concentration and collection were already carried out. This experiment was conducted only to transport the droplet with collected particles into the micro-reactors. The experimental procedure of the particle transport chip is shown in the following:

- 1) Seal air vent, and outlet (Figure 3.23).
- 2) Gently load the particle concentrated droplet into the intake.
- 3) Using syringe pump to move the droplet into the reactors one by one, and take photos of the transport process.

After the experiment, it was concluded that the droplet with the collected particles could be transported into the micro-reactors. The details of the results were discussed in Chapter 4.

3.5. Methods for concentration and collection of *E. coli* cells

Fluorescent particles were used to as the mimic of the bacteria, and lots of experiments were carried out to demonstrate the utilization of the particle concentration and collection on the proposed chips. *E. coli* cells were one of the *E. coli* cells which are commonly found in the environmental samples are often used as the indicator of environmental pollution. Presented here is the simulation of the process of concentrating and collecting *E. coli* cells in the water sample by the proposed chip.

3.5.1. J channel chip design for *E. coli* concentration and collection

The design of the J-channel chip employed a J-channel pattern made of three PMMA layers and J-pattern plastic tubing (1mm inner diameter) (Figure 3.26). The first layer is a substrate which is flat enough to attach on rotating plate, and the second layer is used as a tubing holder to prevent the tubing from falling out of the chip during centrifugation. The third layer holds the tubing and provides a sample loading port of the chip. The tubing provides the J-pattern channel where the cells can be concentrated, and the concentration zone/point is at the bottom of the J-channel.

Figure 3.26 Schematics of the J-channel pattern chip structure.

3.5.2. Working principle of the J-channel chip

The working principle of the J-channel chip in this experiment is the same as the one in Section 3.4.1.2.

3.5.3. The fabrication of the J-channel chip

The illustrations of the three layers in Corel Draw were shown in Figure 3.27. The parameters of the laser setting for 1.5 mm thick PMMA sheets were Power 100%, Speed 8%, PPI 1000 and the parameters of the laser setting for 5 mm thick PMMA sheets were Power 100%, Speed 2%, PPI 500. After the patterns of three layers were generated in PMMA sheets, the vulcanizer was used at the

condition of 127°C for 30 minutes to bond the PMMA sheets together. The photo of the fabricated J-channel pattern chip was shown in Figure 3.28.

Figure 3.27 Layer pattern of J-channel pattern chip. (a) Layer 1: 1.5mm thick; (b) Layer 2: 1.5mm thick; (c) Layer 3: 5mm thick.

Figure 3.28 Photo of fabricated J-channel pattern chip for *E. coli* cell concentration and collection.

3.5.4. Materials

Fluorescence labeled *E. coli* suspension was used as sample suspension. *E. coli* cell is a bacterium shaped like a propane cylinder measuring approximately 2 microns long and 0.8 microns wide. The labeling process for the *E. coli* cells was as follows:

100 μ l over-night *E. coli* culture with the optical density (OD) equal to 0.8 was incubated with 10 μ l 5 mg/ml biotin labeled *anti-E. coli* antibody for 30 min. 10 μ l 0.5mg/ml FTIC (Fluorescein isothiocyanate) conjugated streptevadin was added into the mixture, and incubated in dark for 30 minutes. After that, the *E. coli* cells were labeled successfully. After labeling the cells, we took out 5 μ l from the labeled *E. coli* mixture, and dripped the mixture into 400ml DI-water. Finally, the sample suspension was ready.

3.5.5. Instrument setup

The experimental system and the fluorescence imaging system were the same as the ones used in Section 3.4.1.5.

3.5.6. Characterization of concentrating *E.coli* cells in J channel chip

As described in Section 3.2, the total concentrating efficiency E_T is defined as simply the ratio of the mass of all particles separated (M_c) to the mass of all solids fed into the J channel pattern chip (M). From Equation (3.2) $E_T = M_c/M = 1 - M_s/M$, we can get:

$$E_T = 1 - M_s/M = 1 - \rho Q_s N_s / \rho Q N = 1 - (Q - Q_c) N_s / Q N. \quad (3.6)$$

Since compared with Q , Q_c can be neglected, the total concentrating efficiency E_T can be calculated by:

$$E_T = 1 - (Q - Q_c) N_s / Q N = 1 - Q N_s / Q N = 1 - N_s / N. \quad (3.7)$$

where:

M is the mass of the feed (in kg)

M_c is the mass of the particles concentrated in the J channel pattern chip (in kg)

M_s is the mass of the particles in the supernatant (in kg).

Q is the volume of the feed (in mL)

Q_c is the volume of the fluid in the J channel pattern chip (in mL)

Q_s is the volume of the supernatant (in mL)

ρ is the density of *E. coli* cells (in kg/m³)

N is the *E. coli* cell number in the feed

N_c is the *E. coli* cell number concentrated in the chip.

N_s is the *E. coli* cell number in the supernatant.

In this experiment, the viable plate count method was used for the enumeration of *E. coli* cells.

The working principle of viable plate count is as follows:

The bacteria are poured on to an agar plate for incubation, and each cell will grow into a colony which is visible to the naked eye. Then, if the bacteria are dilute enough, the number of bacteria can be obtained by a count of the number of colonies in the plate (Figure 3.29). Otherwise, the sample of bacteria needs to be diluted first. Usually, the final plates have 30-300 colonies (If the number of the colonies is smaller than 30, it is not acceptable for statistical reasons, and if the number of the colonies is larger than 300, they may not be distinguished from each other.). In this condition, the concentration of cells in original sample can be obtained by counting the number of colonies and multiplying the number by dilution factor [75, 76].

Figure 3.29 Photo of the plate with *E. coli* colonies.

3.5.7. Experimental procedure

The procedure of the *E. coli* concentration and collection experiment is shown in the following:

- 1) Place the J channel chip on the rotating plate.
- 2) Press the button of “Vacuum” to hold the chip on the plate.
- 3) Set rotating parameters such as the rotating time and speed.
- 4) Close the cover of the rotating machine, and begin to rotate the rotating plate.
- 5) Insert the dispensing nozzle into the sample loading port of the chip.
- 6) Start to load the sample by turning on the power supply of the sample loading pump.

- 7) After the rotation stops, observe concentration zone/point to find whether the particles are concentrated successfully.
- 8) If the particles can be observed in the concentration zone/point, gently blow air from the loading sample port to extract supernatant out of the chip.
- 9) After most of the supernatant is extracted, blow air from the supernatant removal port to roll back the particle collected droplet into the concentration zone/point.
- 10) Repeat blowing air from sample loading port to supernatant removal port, and supernatant removal port to sample loading port until all the particles concentrated are collected into the droplet.

After this experiment, it was demonstrated that 100 μ l *E. coli* sample suspension could be concentrated, and the concentrated particles could be easily collected into a moving fluid droplet. The feed rate was 0.2mL/h when the concentrating efficiency reached up to 90%. The details of the results were discussed in Chapter 4.

3.6. Methods for surface treatment of PMMA channels

The particle concentrating efficiency could reach a high level, but the droplet based particle collection efficiency were still need to be promoted. Several papers and patents were reported that poly (ethylene oxide) (PEO) based

polymers could be deposited on the surfaces to effectively inhibit the cell adhesion [72-76]. We treated PMMA sheets with PluronicTM F108 (BASF, Germany) solutions in the different concentrations to investigate the relationship between Pluronic F108 concentration and cell detachment.

3.6.1. Chip design and fabrication

As shown in Figure 3.30, our design employed seven 2mm*3mm channels. The chip was made of three PMMA layers. The first layer was a substrate which was used to enclose the channel, and the second layer was the channel layer in which the Pluronic F108 would flow through, and generate a thin film. The third layer was used to provide the connection between the chip and tubing.

Figure 3.30 Schematic drawing of the surface treatment testing chip.

The illustrations of the three layers in Corel Draw were shown in Figure 3.31. The parameters of the laser setting for 1mm thick PMMA sheets were Power 40%, Speed 8%, PPI 1000 and the parameters of the laser setting for 3 mm thick PMMA sheets were Power 90%, Speed 5%, PPI 500. After the patterns of three layers were generated in PMMA sheets, the vulcanizer was used at the condition of 127°C for 30 minutes to bond the PMMA sheets together. The photo of the fabricated surface treatment testing chip was shown in Figure 3.32.

Figure 3.31 Layer pattern of the surface treatment testing chip. (a) channel layer: 3mm; (b) top layer: 1mm; (c) bottom layer: 1mm.

Figure 3.32 Photo of the fabricated surface treatment testing chip.

3.6.2. Surface treatment

PluronicTM F108 (BASF, #F108) was selected as the chemical to treat the channel wall. Solutions of 1% to 6% w/w PluronicTM F108 in water were prepared, and injected into the channel in the chip. The solutions stayed in the channel for 24 hours at room temperature for absorption. After that, fresh water was pumped into the channel to extract the rest of the solutions and clean the chip.

3.6.3. Characterization of surface treatment of PMMA channels

To quantify particle adhesion on PluronicTM F108-treated surfaces, yellow green fluorescent beads with a 1 μm diameter (Product No. SKU#F-8823, Invitrogen Corp. Eugene, Oregon, CA, USA specified absorption wavelength 505 nm,

specified emission wavelength 515 nm) were diluted 1:500 in pure water, and placed into a 15 ml plastic tube as the sample suspension. The sample suspension flew through the PluronicTM F108-treated channel (channel 0-6), and collected into a tube. The efficiency of the particle adhesion could be calculated by the method used in Section 3.4.1.6.

In this experiment, it was demonstrated that the influence of PluronicTM F108-treated PMMA surfaces on the fluorescent beads adhesion was tiny. The influence of the adhesion of the fluorescent beads on the PluronicTM F108-treated PMMA surfaces could be neglected. The details of the results were discussed in Chapter 4.

Chapter 4 Results and discussions

This chapter described and discussed the results of the experiments discussed in Chapter 4. Thus, this chapter was structured according to the sequence of Chapter 4. At first, the results of concentrating, collecting and transporting the fluorescent beads were presented. Subsequently, the results of concentrating and collecting the *E. coli* cells were reported. Finally, the fluorescent beads adhesion on PluronicTM F108-treated PMMA surfaces was investigated.

4.1. Results and discussions for concentration, collection and transport of fluorescent beads

Two types of chips, J-channel chip and spiral channel chip, were discussed in this section. The capabilities of concentrating, collecting and transporting fluorescent beads were the main elements to be taken into consideration.

4.1.1. The concentration of fluorescent beads

As described in Section 2.1.2.1, centrifugal force can move the more dense components away from the axis, and move the less dense components towards the axis. In this experiment, centrifugal force was used to trap the more dense fluorescent beads, and remove the less dense water away from the chip. A large feed rate of the sample with high concentrating efficiency is the goal for our

research. The relationship between the feed rate of the sample and concentrating efficiency was studied.

4.1.1.1. J-channel chip

The experiments were carried out at the rotating speed of 5000 RPM and 4000 RPM, respectively. The concentrating efficiency was measured in different feed rates. To rule out the artificial error, the experiments in each feed rate repeated for three times. The results including the feed rate, concentrating efficiency were listed in Table 5.1. The drawing to describe the relationship between concentrating efficiency of fluorescent beads and the feed rate at the rotating speed of 5000 rpm and 4000 rpm was shown in Figure 4.1. In the table, it is found out that the concentrating efficiency at the rotating speed of 5000 RPM ranged between 50% and 93% with a trend for higher concentrating efficiency with a lower feed rate (feed rate between 5-20 mL/h), and the concentrating efficiency at the rotating speed of 4000 RPM ranged between 87% and 32% with a trend for higher concentrating efficiency with a lower feed rate (feed rate between 5-20 mL/h). Furthermore, it also shows that a higher rotating speed leads to higher concentrating efficiency.

Table 5.1: Concentrating efficiency in J channel chip with different feed rate at the rotating speed of 5000 rpm and 4000 rpm.

Speed of the rotor(RPM)		Feed rate(ml/h)				
		5	10	13	15	20
5000	First test	0.916	0.736	0.626	0.629	0.476
	Second test	0.937	0.815	0.650	0.628	0.526
	Third test	0.951	0.796	0.755	0.602	0.511
4000	First test	0.867	0.644	0.611	0.450	0.426
	Second test	0.865	0.636	0.601	0.493	0.209
	Third test	0.871	0.579	0.513	0.385	0.325

Figure 4.1 Concentrating efficiency in J channel chip with different feed rate at the rotating speed of 5000 rpm and 4000 rpm.

4.1.1.2. Spiral channel chip

The experiments were carried out at the rotating speed of 5000RPM. Like the measurement in the J-channel chip, the concentrating efficiency was measured in different feed rates. The experiments in each feed rate also repeated for three times. The results including the feed rate, concentrating efficiency were listed in Table 5.2. The drawing to describe the relationship between concentrating efficiency of fluorescent beads and the feed rate at the rotating speed of 5000 rpm was shown in Figure 4.2. In the table, it is found that the concentrating efficiency at the rotating speed of 5000 RPM ranged between 50% and 100% with a trend for higher concentrating efficiency with a lower feed rate (feed rate between 60-180 mL/h).

Table 5.2: Concentrating efficiency in Spiral channel chip with different feed rate at the rotating speed of 5000 rpm.

Speed of the rotor(RPM)/Feed rate(mL/h)		60	90	120	150	180
5000	First test	0.998	0.992	0.769	0.641	0.537
	Second test	0.999	0.994	0.770	0.543	0.595
	Third test	0.998	0.992	0.780	0.593	0.504

Figure 4.2 Concentrating efficiency in spiral channel chip with different feed rate at the rotating speed of 5000 rpm.

4.1.1.3. Comparison between J-channel chip and spiral channel chip

The concentrating efficiencies of both J-pattern chip and spiral chip at the rotating speed of 5000 RPM were shown in Figure 4.3. It is demonstrated that comparing with the J channel chip at the same concentrating efficiency the spiral chip had a much larger feed rate. It also is concluded that spiral chip is much more suitable than J-channel chip to concentrate the particles in the environmental sample which usually had a large volume.

Figure 4.3 The relationship between the feed rate and the concentrating efficiency of both J channel chip and spiral channel chip.

4.1.2. The collection of fluorescent beads

After the concentration process, the fluorescent beads were concentrated inside the channel. Then, it is very important to collect the fluorescent beads for further analysis. In this experiment, a water droplet was introduced to move through the concentration zone where fluorescent beads were concentrated. The processes of particle concentration and collection in the J-channel chip were traced in the fluorescence imaging system. Firstly, empty J-channel chip was placed into the fluorescence imaging system. Then fluorescence labeled *E. coli* sample was loaded into the J-channel chip from the sample loading port (Figure

4.4 (a)). The chip was placed into the spin processor, and kept spinning at 5000 rpm with the feed rate of 10 mL/h (Figure 4.4 (b)). After that, the supernatant was extracted from the supernatant removal port by air pumping (Figure 4.4 (c)). The droplet was rolled back to collect the rest of fluorescent beads (Figure 4.4 (d)-(h)). Finally, the droplet with collected particle was transported out of the chip for further analysis.

(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)

Figure 4.4 The particle concentration and collection procedure in J-channel chip. (a) load sample into the chip; (b) Spin the chip to concentrate particles; (c) pump a water droplet into the channel to collect particles; (d-h) pump air to move the droplet forward and back to collect the rest particles.

4.1.3. The transport of fluorescent beads

After fluorescent beads are collected into the water droplet, it was also important to transport the droplet to the downstream functional modules. In this experiment, the droplet was transported to the array of micro-reactors. The integrated particle transport chip used in this experiment was illustrated in Section 3.3.3. The transporting process was shown in Figure 4.5. Firstly, the sample was loaded into the chip from the loading sample port (Figure 4.5 (a)), and then air was gently pumped into the chip to move the sample to the concentration zone/point. Then, the waste port was sealed and air was pumped from the sample loading port to move the sample towards the array of micro-reactors (Figure 4.5(b)-(d)). At last, the sample was transported into the array of micro-reactors easily.

(a)

(b)

(c)

(d)

Figure 4.5 Image sequence for distribution of concentrated droplet into an array of micro-reactors for real-time quantitative micro-PCR on-chip. (a) load the sample into the chip from the sample loading port; (b) seal the waste port, and gently pump air through the air tubing into the channel to move the sample; (c) keep pumping air into the tube, make sure that the sample will be gradually loaded into the microarrays; (d) seal the air vents, and open the absorbent to pump out the supernatant.

4.2. Results and discussions for concentration, collection of *E. coli* cells

Fluorescent particles were used to as the mimic of the bacteria, and lots of experiments were carried out to demonstrate the utilization of the particle concentration and collection. Although the size of the fluorescent beads is nearly the same as most bacteria, it was still necessary to concentrating and collecting a type of real bacterium in the proposed chip. In this experiment, *E. coli* cells were used as the sample to be concentrated and collected.

4.2.1. The Concentration of *E. coli* cells

The experiments were carried out at the rotating speed of 5000RPM. The concentrating efficiency was measured in different feed rates. The results including the feed rate, concentrating efficiency were listed in Table 5.3. The

drawing to describe the relationship between concentrating efficiency of *E. coli* cells and the feed rate at the rotating speed of 5000 rpm was shown in Figure 4.6. In the table, it was found that the concentrating efficiency at the rotating speed of 5000 RPM ranged between 63% and 98% with a trend for higher concentrating efficiency with a lower feed rate (feed rate between 10-50 mL/h).

Table 5.3: Concentrating efficiency of *E. coli* cells in J channel chip with different feed rate at the rotating speed of 5000 rpm.

Feed rate(ml/h)	10	20	25	30	35	45	50
<i>E. coli</i> cell number in the supernatant	3	6	12	23	37	51	59
Total <i>E. coli</i> cell number	161	161	161	161	161	161	161
Concentrating efficiency	0.981	0.963	0.925	0.857	0.770	0.683	0.633

Figure 4.6 Concentrating efficiency of *E. coli* cells in J channel chip with different feed rate at the rotating speed of 5000 rpm.

This experiment demonstrated that *E. coli* cells could be continuously concentrated in our proposed chip by the centrifugal force.

4.2.2. The collection of *E. coli* cells

After the concentration process, the *E. coli* cells were concentrated inside the channel. Then, it is very important to collect the *E. coli* cells for further analysis. In this experiment, a water droplet was introduced to move through the concentration zone where *E. coli* cells were concentrated. The processes of cell concentration and collection in the J-channel chip were traced in the

fluorescence imaging system. Firstly, empty J-channel chip was placed into the fluorescence imaging system. Then fluorescence labeled *E. coli* sample was loaded into the J-channel chip from the sample loading port (Figure 4.7(a)). The chip was placed into the spin processor, and kept spinning at 5000 rpm with the feed rate of 10 mL/h (Figure 4.17 (b) (c)). After that, the supernatant was extracted from the supernatant removal port by air pumping (Figure 4.17 (d), (e), (f)). The droplet was rolled back to collect the rest of fluorescent beads (Figure 4.17 (g), (h), (i)). Finally, the droplet with collected cells was transported out of the chip for further analysis.

Figure 4.7 The particle concentration and droplet based collection process. (a) the schematic of channel position; (b) Empty J-channel pattern chip; (c) Load fluorescence labeled *E. coli*; (d) Spin at 4500 rpm for 30 minutes (e-g) Extract supernatant from the supernatant removal port; (h-j) Roll the droplet back to collect the rest of cells.

This experiment demonstrated that the concentrated *E. coli* cells discussed in section 3.5 could be collected into a water droplet for further analysis.

4.3. Results and discussions for surface treatment of PMMA channel

The experiments discussed above demonstrated that both fluorescent beads and *E. coli* cells could be continuously concentrated and collected into a droplet for further analysis. The total efficiency can be calculated by the concentrating efficiency multiply by the collecting efficiency, where the concentrating efficiency is the ratio between the number of the concentrated particles and the number of the feed particles, and the collecting efficiency is the ratio between the number of the particles collected into the droplet and the number of the concentrated particles. As discussed before, the concentrating efficiency could reach up to nearly 100%. However, the collecting efficiency was still not high enough. This experiment was expected to improve the collecting efficiency by treating PMMA channel wall with PluronicTM F108 solutions. In this experiment,

each channel of the multi-channel chip was treated by the solutions of 0%-6% w/w PluronicTM F108 in water. However, the experiment showed that the concentration of the PluronicTM F108 solutions did not play an important role on the collecting efficiency. A method to promote the particle or cell detachment in the channel wall is needed.

Chapter 5 Conclusions

In this work, the micro-fabrication technology such as laser cutting was used in the chip fabrication, which made the fabrication process easier and more time-saving. The fluorescent beads as mimic of the bacteria in the water were continuously concentrated by the centrifugal force. The fluorescent beads and the *E. coli* cells were both continuously concentrated in the Point Capture chip and the Distributed Surface Capture chip. The J channel chip which was one type of the Point Capture chip had the feed rate of 5-20mL/h with the concentrating efficiency of 93%-50% in fluorescent beads concentration, and the spiral channel chip which was one type of the Distributed Surface Capture chip had the feed rate of 60-180mL/h with the concentrating efficiency of 100%-50% in fluorescent beads concentration. For the low throughput, the proposed J channel chip could only be used to monitor the break out of water contaminations, and the proposed spiral channel chip which had a larger feed rate could be used to water microbe detection. The particle concentration process by the continuous centrifugation was much simpler than the particle concentration process in other ways, such as filtration, magnetic activated cell sorting, dielectrophoresis, etc. PDMS deposition in the channel of the chip was used to decrease the toughness of the channel so that the concentrated particles would be collected into the droplet more easily. An integrated particle transport chip was developed to concentrate particles in the Point Capture channel, collect particles into a DI-water droplet, and transport particles into downstream functional modules, such as a micro-PCR reactor chip. It was demonstrated that

a device with the functions of sample pre-concentration, DNA extraction, and PCR analysis could be developed and applied to water environmental industry.

Based on the work done, the following conclusions can be drawn:

- 1) Based on micro-fabrication technology such as laser cutting, the prototypes of chips are fabricated, and this technology is time saving and easy to carry out.
- 2) Based on the experiments of the Point Capture chip (Section 3.4.1), it was demonstrated that fluorescent beads can be concentrated in the concentration zone/point and collected by a droplet. This design provides a new way to concentrate, collect and transport particles for particle analysis.
- 3) Based on the experiments of the Distributed Surface Capture chip (Section 3.4.2), the conclusion is made that compared with the Point Capture chip, the Distributed Surface Capture chip can concentrate fluorescent beads at a larger feeding flow rate if they have the same concentrating efficiency.
- 4) Based on the experiments of the integrated particle transport chip (Section 3.4.3), the feasibility to transport concentrated particles in the

channel into downstream functional modules such as the array of micro-reactors was demonstrated.

- 5) Based on the experiments in Section 3.5, the conclusion is made that *E. coli* cells can be continuously concentrated and collected in our proposed chip by the centrifugal force.
- 6) Based on the experiments of surface treatment of PMMA channels in Section 3.6, the PluronicTM F108 (BASF, #F108) which can be used to treat Polystyrene sheets (PS) does not work on PMMA sheets.

In summary, it was concluded that both fluorescent beads and *E.coli* cells could be continuously concentrated and collected by our proposed chips. Further more, the concentrating efficiency of the spiral chip in this case was higher than that of the J-channel chip. The continuous centrifugation technology can be used in environmental sample concentration.

Chapter 6 Improvement and future work

Two methods of continuous flow centrifugation, namely the Point Capture method and the Distributed Surface Capture method, were presented in Chapter 3, and the feasibility of the droplet based particle collection was demonstrated in the experiments. However, the parameters of chip design such as chip size, channel height, channel width, are still needed to be optimized so that the feed rate can reach a high feed rate with a high concentrating efficiency. Moreover, although most of the concentrated particles can be collected by a DI-water droplet, some particles still attach on the channel wall. To make a more accurate detection, a method to promote the particle detachment of the channel wall is required. Two methods can be used to improve the bacteria detachment of the channel wall. The first one is to decrease the bacterial attachment by modifying the ionic strength. This method has been investigated in the research of bacterial transport long time ago. As early as 1991, Fontes et al. [77] reported that ionic strength of the cell suspension had the effect on the transport of cell through porous media, which also indicated that we could improve the particle collection by modifying the ionic strength of the droplets used to collect particles. The other method is to apply an advanced surface treatment on the channel wall to decrease the bacterial attachment. Lei Shi [78] et al. found that the mucin coating on polymeric material surfaces can suppress bacterial adhesion. Further more, the rotating speed of the spin processor can also be increased to get a higher feed rate.

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Appendix 1: Particle information in Sigma-Aldrich

Micro particles based on melamine resin, carboxylate-modified, FITC-marked				
Product number	Size	Solid concentration	Fluorescence	Estimated delivery date
86296	1 μm (Sigma)	2.5%	λ_{ex} 490 nm; λ_{em} 525	Feb, 10
88486	3 μm (Fluka)	2.5%	λ_{ex} 490 nm; λ_{em} 525	Feb, 10
88901	6 μm (Fluka)	2.5%	λ_{ex} 490 nm; λ_{em} 525	Feb, 10
90287	10 μm (Fluka)	2.5%	λ_{ex} 490 nm; λ_{em} 525	Feb, 10