

FLUIDICS

- THE LINK BETWEEN MICRO AND NANO SCIENCES AND TECHNOLOGIES -

Chih-Ming Ho

Mechanical & Aerospace Engineering, University of California,
Los Angeles, USA 90095-1597

Email: chihming@ucla.edu, <http://ho.seas.ucla.edu>

ABSTRACT

Micro fluidics is a collection of processes for moving bulk fluid mass or controlling the paths of selected embedded particles, cells or molecules, in flows. Length scale matching between the flow and the device is the key for efficient momentum and energy transfers of the desired fluid motions. MEMS enable us to handle minute amount of fluid in the nano or pico liter range. With properly designed micro fluidic devices, molecules can be directly manipulated by the flow patterns inside the device, which provides a pathway to exploit the nano world. Obviously, understanding of the molecular effects on flows becomes a crucial issue. In traditional fluid dynamics, the flow length scale is much larger than the molecular length scale. Continuum is the most common hypothesis for flow researches. In the case of micro/nano engineering system, we are in the transition regime between continuum and molecule dominated conditions. This feature brings us the challenges when exploring the science and developing the technology in micro/nano fluidics.

INTRODUCTION

For most of the bio-medical analyses, the first step is to collect cells, which may be either airborne, in water or in the tissue. These samples will then be processed in liquid by introducing various reagents. When intra-cellular materials are interested, cells need to be lysed and more reagents in liquid form will be added in order to obtain detection signal. In these processes, fluid serves as the medium to transport cells, proteins, DNA/RNA and particles from one location to another place in the detection system. In the case where reactions need to take place, the molecules or probes in reagents are required to be mixed with target cells or DNA/RNA. In other cases, we need to separate species from each other. These fluidic processes are generic in bio-medical analyses whose specificity, sensitivity and time required for accomplishing the process are the key requirements. The bulk fluid transport and handling motion of the embedded molecules play crucial roles in determining the processing time and sensitivity. Obviously, the specificity and sensitivity are primarily decided by the sensor design.

In most cases, length scale matching between the transducer and the phenomenon to be studied is a necessity for efficient couplings. The couplings include pick, place, straining as well as momentum and energy transfers, etc. With the continuing improvement of the spatial resolution of transducers by micromachining technology, it is possible to directly handle a single cell, which is on the order of microns. Kim et al [1] has first demonstrated a micro mechanical gripper that can hold a single

euglena with a diameter of 7 microns. Recently, Kim's group [2] developed a cell cage that can be operated in liquid mediums. The cell is the basic element of living organisms while cellular researches are always carried out in aqueous environment. Therefore, fluidic processes in micro configurations emerge as a critical discipline in the bio-medical field.

Micro/nano flows are very different from the macro flows. Continuum is commonly assumed in macro flows. Molecular effects are represented by physical constants such as viscosity. In macro flows, the inertia of fluid mass is usually much larger than the viscous force hence the large Reynolds number. Most interesting phenomena are the manifestations of non-linear effects associated with the inertial forces. In micro devices, the typical Reynolds number is much less than one due to the small transverse length scale, which results in high velocity gradient and thus high viscous force. Low Reynolds flows, Stokes flows, are linear in nature. However, linearity may not guaranty a simple solution, especially when the scale of the micro flows is not too much larger than the sizes of macro-molecules such as DNA/RNA or protein. In other words, the basic hypothesis, continuum, breaks down. Furthermore, due to the large surface to volume ratio in micro devices, these macromolecules are not very far from the surface and the motions are influenced by the surface potential, which leads to the not very well understood region of size effects. These intriguing molecular effects in micro fluidics leads to new direction for fundamental fluid mechanics research in addition to numerous applications in bio-medical engineering area. It is interesting to note that these features result only from the matching between molecular length scale and device length. Except in few special nano/micro flow conditions, we have not observed the importance of molecular time scale because it is typically much smaller than the time scales of slow moving micro flows. In the other transport phenomena, micro/nano heat transfer, the time scale effects shares the equal role with the length scale effects [3].

Hydrodynamic pressure is the primary force field for driving the fluid in macro flows and still frequently used in driving bulk fluid in MEMS. However, the pressures drop increases with the second power of the transverse dimension and can be too large in micro passages to become impractical. Electrokinetic (EK) force fields functions efficiently near a surface, e.g. electroosmotic (EO) force or dielectrophoretic (DEP) force, or in an electric potential field, e.g. electrophoretic force. EK forces are commonly used in micro fluidic systems. With proper design, the micro flow pattern activated by either hydrodynamic pressure or EK forces can directly manipulate the motion of molecules. Hence, micro flows can serve as a link between micro and nano technologies.

Micro fluidics is to understand the underlying physical mechanics of the molecule or bulk fluid motions in micro/nano configuration and to achieve efficient designs for facilitating the processes used in bio-medical/chemical analyses. In this paper, the fundamental issues such as role of molecules in viscosity and the force fields for driving molecules are reviewed first. We then discuss the fluidic processes of directly manipulating macro molecule, DNA, by micro fluidics, sample concentration, mixing, separation, as well as molecular recognition for bio-detections.

VISCOSITY

Viscosity is a physical property derived under the hypothesis of continuum. It represents the tendency of a fluid to undergo deformation when subject to a shear stress. The deformation rate is determined by the inter-molecular force, which provides the force to balance the applied shear force. In fact, viscosity is a global material constant or constants representing the propensity for a collection of fluid molecules to externally applied stress.

In the case of a pure liquid consisting of simple molecules, viscosity is thought to be an intrinsic property and its value is dependent on the temperature of the fluid alone. For more complex fluids, such as solutions of macromolecules, viscosity may contain more than one constant and generally shows a complex dependence on an additional number of parameters, such as type of solvent, or concentration, molecular weight, conformation, etc of the solute. Nonetheless, even in these more complex cases, viscosity is ascribed to the intrinsic properties of the fluid itself [4].

When the length scale of the flow field becomes close to the size of the molecules of the liquid, the underlying assumption that the fluid can be described as a continuum is not valid. The "intrinsic" viscosity shows a departure from their typical values. When this happens the concept of viscosity must be reconsidered and reinterpreted as "apparent" viscosity in continuum term. This phenomenon is actually caused by "molecular effects". Experimental evidence on flows in molecular size channel shows how dramatic the molecular effect is as the size of the channel and the size of the molecules "flowing" in it become comparable. For example, when the channel size is below ten molecular diameters, in the order of a few nanometers, the fluid loses its liquid-like behavior and assumes solid-like characteristics [5]. The structure of the molecules in the molecular size channels changes from a random order to a discrete number of ordered layers, with the "apparent" viscosity as high as 10^5 times the "regular" viscosity and the typical molecular timescale of the fluid 10^{10} times slower [6]. Figure 1 represents the results of a typical experiment where two parallel plates, separated by a few molecular layers of OMCTS (octamethylcyclotetrasiloxane), sliding with respect to each other, while the distance between plates decreases with time (horizontal axis in Fig. 1). There are distinctive features of this sort of molecular flow: (1) the frictional force increases almost in a stepwise fashion as the distance between the plates is reduced, and reaches a stable value when the separation consists of an integer number of molecular layers (1, 2 or 3); (2) the sawtooth behavior is due to a stick-stick motion of the plates coincident with the rearrangement of the molecular layers moving with respect to the other.

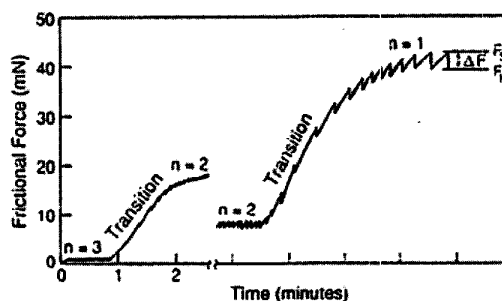


Figure 1. Friction force between two parallel plates

In the case of solutions of macromolecules, such as DNA in most of biological solutions, the contour length of DNA macromolecules can be of tens of microns and their persistence lengths on the order of one micron. The molecular effect can be apparent for much larger channels, a few microns or even larger, despite the fact that the solvent by itself behaves like a continuum, as its molecular size is 4 or 5 orders of magnitude smaller than the channel.

In the case of a dilute solution, even the interaction between macromolecules can be negligible. The interference of the DNA molecule with the wall can be significant for channels of one micron or so. The "wall effect" can be predominant in MEMS devices due to the large surface to volume ratio. This is due to the physicochemical interaction between fluid molecules and wall molecules. Its extension is not necessarily confined to the first layer, or the first few layers, of molecules lying in contact with the wall, but can propagate across the whole flow field. For example, fluid molecules structure themselves at the wall in a different fashion depending on its hydrophilic or hydrophobic nature. In the presence of flow, anomalous phenomena are observed for the case of a hydrophobic surface, and they are generally explained with the so-called "wall slip". Another example is the phenomenon of "wall depletion" of polymer in a dilute polymer solution. The polymer concentration across the flow field presents a "depleted sub-layer" at the wall [7]. Wall effects are the result of competing forces that create a different state of equilibrium within the fluid. In the latter case, for example, the electrostatic repulsive force caused by the wall competes with the osmotic force generated by the non-uniform distribution of solute in the bulk of the fluid. Hence, the polymer concentration is neither uniform, nor the polymer is completely relegated to the center of the channel; instead, a finite concentration gradient forms across the channel. As in the case of the molecular effect, the wall effect is related to the ratio of two quantities. In the former case, it was the ratio of two characteristic lengths (molecular size versus channel size); in the latter, it is the ratio between surface forces and volume forces. In principle, surface forces scale with the second power of the characteristic length of the flow, while volume forces scale with the third power. Their ratio is inversely proportional to the characteristic length of the flow, so that, when the ratio between volume forces and surface forces is small enough, body force are negligible, and the behavior of the flow depends on the size. In general, it is reasonable to expect that surface induced phenomena depend on some power of the flow characteristic length that is negative, even

though not necessarily equal to -1 . Wall effects can take place at much larger length scales of the flow than geometrical effects: for example both slip of simple fluids and of polymer solutions is observed in tube of several millimeters in diameters. Not much work is available to predict the behavior of such solutions in this size range and the studies performed to date are yet far from providing a clear and consistent picture of the size effect. This offers the opportunity to open a new area of the research in the micro fluidics field with both extreme scientific interest and definite practical relevance.

The dramatic change of properties of the fluid with the scale of the flow has a great impact on the design and applications of MEMS. The hydraulic power required to perform a certain process is equal to the product of the total pressure drop sustained during the process and the flow rate. Pressure drop and flow rate relates to each other by the value of the "apparent" viscosity and, as such, they can be strongly affected by the size of the device. In similar fashion, for applications where the control of flow rate is critical (drug delivery, biomedical devices, etc.), the value of the "apparent" viscosity pertaining to the size of that MEMS device must be known in order to correctly determine the actual amount of fluid being dispensed.

ELECTROKINETIC FORCE FIELDS

MEMS technology makes the integration of electrodes in micro reactors/channels become a reasonably simple procedure. Hence, EK forces, which are more effective in small dimensions, are commonly used in micro fluidics.

Electro-osmosis describes the motion of an ionic fluid induced by an external DC potential applied along the stream. The electrical field exerts a net electrostatic force on the ions, which then drag the solvent along. Electro-osmosis can also occur in a neutral liquid due to the electrical double layer formed at the solid-fluid interface. A solid surface in contact with a liquid experiences a surface charging mechanism through two possible possibilities:

- surface molecules dissociate (protons leave the surface which hence becomes negatively charged).
- ions from the liquid bound onto the surface.

The charged surface is then balanced by two populations of counterions, whose total charge equals the surface charge. (see Fig. 2)

- some ions transiently bound to the surface and form the Stern or Helmholtz layer.
- some ions form are in a rapid thermal motion close to the surface and form the diffuse electric double layer.

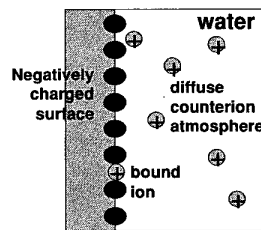


Figure 2 Electrical double layer principle

The diffuse electric double layer acts as a charged capacitor, whose equivalent plates separation is known as the Debye length $1/\kappa$. It depends solely on the properties of the liquid and ranges from nm for high ionic concentration solutions to μm range ($1/\kappa \approx 1\mu\text{m}$ for DI water). If a

potential is applied along the channel, the diffuse electric double layer will move due to electrostatic force. This means the fluid boundary layer is subject to a net force and moves, dragging along the rest of the fluid. The velocity profile is therefore constant across the channel.

Several other types of EK forces are useful in moving the embedded particles, cells, and molecules relative to the solvent. The electrophoretic force is for moving charged particle embedded in neutral medium. The particle of charge q subject to an external electric field E experiences an electrostatic force $F=qE$. An electrical field of 10^6 V/m (equivalent to 10V over a $10\mu\text{m}$ gap), yields a force of $1.6 \cdot 10^{-13}$ N on a single charged particle. Thus, with a friction coefficient given f by Stokes's law $f = 6\pi r\mu$, where r is the particle radius and μ the medium viscosity, the particle is given a velocity $v = \frac{q}{6\pi r\mu} E$. We should note that the

viscosity, μ , might not be the nominal bulk viscosity for the reasons discussed in the previous section. For micron size particles in water, it yields velocities in the order of $20\mu\text{m/s}$. For a uniform electric field, the force is constant over distance, and does not depend on the properties of the particles, only its charge.

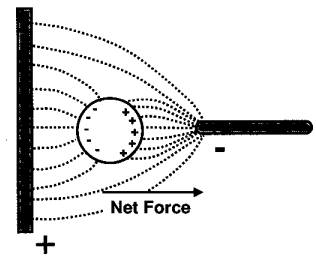


Figure 3 Positive DEP force.

A redistribution of surface charges of a neutral particle can occur when an external electric field is applied to polarize the particle. If the electric field is not spatially uniform, the local distribution of charges on the particle will induce local dielectrophoretic (DEP) forces $dF(x)=dq \cdot E(x)$ [8]. Although the net charge is zero, the net force can be non-zero since the local forces are different. The particle will then move towards the lowest or the highest field gradient regions, according to its polarization. An example of positive dielectrophoresis is given in figure 3.

Polarization is needed to move charges around the particle therefore DEP force is frequency dependent, when the applied electric field is an AC voltage. Both the magnitude and the direction of

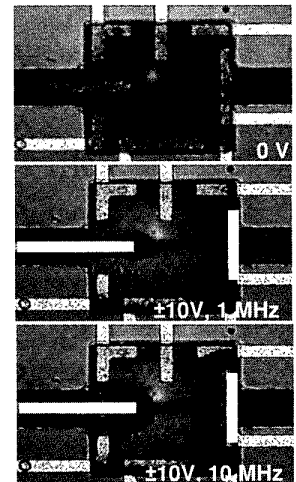


Figure 4 Collection and repulsion of polystyrene particles.

the force are frequency dependence through the Clausius-Mossotti factor. The direction of force depends on the difference between the complex permittivities of both the particle and the medium. The magnitude of the force is proportional to the second power of

the electrical field gradient and the third power of the particle diameter. In the negative DEP force domain, particles are repelled from high electric field gradient, typically on electrode edges. Positive DEP provides an attraction force. Figures 4 shows results of experiments performed with 0.505 μm polystyrene particles in aqueous medium ($\sigma=1.28\text{mS/cm}$), in a cavity of 200x200x25 μm . Typical DEP forces are on the order of 10^{-16}N per particle. Note that this force varies widely in space as it depends on the square of the gradient of the electrical field.

Recently, extensive publications on dielectrophoresis applications to particles manipulation and separation became available ([9], [10], [11]). Particle can be separated based on their surface properties. Green and Morgan [12] demonstrated the separation of nano particles by DEP force (Fig. 5). The initial heterogeneous mixture is composed of 93 nm diameter fluorescently loaded latex beads. Beads with lower surface

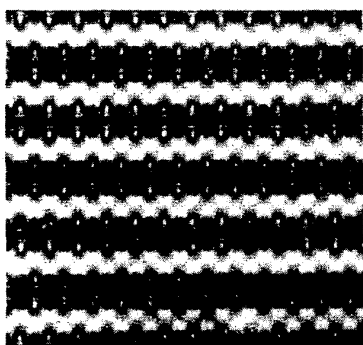


Figure 5. Separation of particles of different surface density charge.

charge move to the low-field regions between the electrode fingers, whilst the beads with the higher surface charge are trapped at the electrode tips.

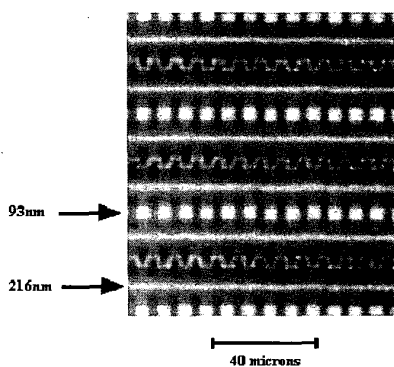


Fig.6 Separation of particles of different surface density charge

By taking advantage of local fluid motions, it is possible to separate particles based on their size [13]. Local drag combined

with different DEP force magnitudes lead to the separation of 93nm particles from 216nm particles, as shown in Figure 6. For a given frequency, the largest particles experience a relatively lower positive DEP force than the smaller particles, due to different DEP domains. Local fluid motion drags them on the top of the electrodes, while the small particles remain collected at the electrode edge.

DIRECT MANIPULATION OF DNA BY HYDRODYNAMIC FIELD

In micro fluidic system, hydrodynamic pressure can serve as a driving force for moving bulk fluids in devices with transverse dimension of 100 microns. The hydrodynamic pressure is usually produced by surface with positive displacement such as a piston and can provide an accurate re-producible flow rate. A MEMS based peristaltic pump is shown in Fig. 7 [14]. On the other hand, the pressure drop

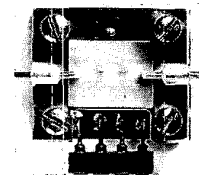


Fig. 7. peristaltic pump

increases with the second power of the transverse dimension of the device. If the velocity is kept the same, a much less constraint condition than constant flow rate, the pressure drop increases four orders of magnitude for devices with dimension of 1 micron and become impractical.

By proper microchannel design, hydrodynamic force is able to produce desired flow field, which can control the deformation of embedded macro molecules. A DNA molecule can be stretched and return to its equilibrium coiled state when the hydrodynamic force is removed. Several groups [15,16] have reported the study of single DNA molecules under shear. Babcock et al. [17,18] made detail description on single DNA dynamics under shear generated between two parallel glass plates. Non-periodic fluctuations in extension and end-over-end tumbling were observed under steady shear. The rate of fluctuation and average extension increased as the shear rate increased.

Another way of stretching a DNA molecule is to use accelerating flow, whose velocity is increasing in the streamwise direction. We have designed a micro fluidic device (Fig. 8), which has three inlets and a converging section, for manipulating T2 DNAs. The two side inlets and the converging section provide shear force to symmetrically accelerate the flow exiting from the center channel, similar to a cytometer but in micro scale. An accelerating flow along the streamwise direction was verified by micro particle image velocimetry and stretching of DNA molecules was demonstrated (Fig. 9). If we stop the flow, the stretched DNA will coil back. The relaxation time is governed by the force balance between DNA elasticity and hydrodynamic drag and can be measured from Fig.10. Perkins et al. [19] used another technique to measure the relaxation of single λ DNA. One end of the DNA was chemically attached to a 1- μm -bead for anchoring with laser

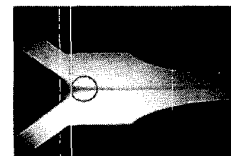


Fig. 8. A channel with accelerating flow

tweezers. Uniform flow was applied around the stationary bead; the DNA was stretched to its full extension due to hydrodynamic drag force. The relaxation was observed after the flow was stopped.

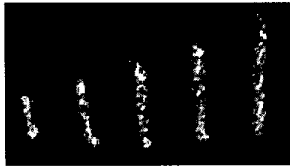


Fig. 9. DNA stretching under accelerating flow.

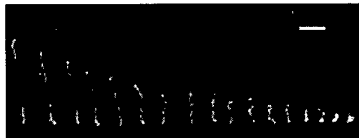


Fig. 10. DNA Relaxation after elongation.

MICRO FLUIDIC PROCESSES FOR BIO-ANALYSES

Sample Concentration

Extracting samples in very diluted concentrate from large amount liquid or air is usually required. Centrifugal force is commonly used to concentrate samples on laboratory bench top. Centrifuge needs large rotation arm and high rotation frequency. Both requirements are difficult to meet in MEMS. For MEMS based bio-analysis system, sample concentration is the interface between macro and micro worlds. In fact, most of the power consumption in the whole system is spent at this step. Efficient micro concentration devices other than the centrifugal force based approach are in need.

Among the others, membrane with perforations (filter) is a candidate for sample collection [20,21]. By passing liquid or air through the micromachined membrane as shown in Figure 11[22],

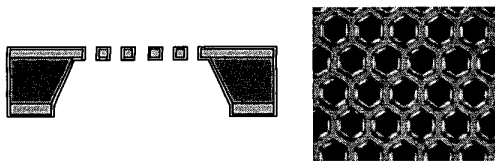


Figure 11. Micromachined particle filter

target particle or biological agents can be collected. For filtering micron size particles, the membrane must be mechanically robust for withstanding high-pressure drop. In order to improve filter strength, a layer of ParyleneC® is conformally deposited on filters. The burst pressure of the filter increases up to 4.2 psi, which is four times more than that of an uncoated filter. With the configurations of 1~3 μm thickness and 4~12 μm hole size, data were collected from many filter designs with opening ratio ranging from 4% to 45%. Based on the collected data, we developed an empirical design rule for predicting the pressure drop [23, 24];

$$K = \frac{2\Delta P}{\rho U_{in}^2} = \beta^{-2} \left[3.5 \frac{t}{d} + 3 \right] \left[10 \frac{\nu}{U_h d} + 0.22 \right]$$

where U_{in} is the velocity in front of the filter, U_h the velocity through the hole with the diameter of d , t the thickness, β the opening ratio, ρ the density of fluid and ν the viscosity. Low pressure drop requires high opening factor (ratio of hole area/ total area), which allows a large amount of fluid passing holes at low velocity.

After particles are collected, the concentrated samples need to be transported into liquid phase for sample preparation. Dielectrophoretic force generated by electrodes deposited on top of the filter is used to move the particles [25, 26]. The dielectrophoretic particle transportation was realized by using a 3-phase traveling voltage. In this system, the dielectrophoretic force acts for particle transportation while adhesion forces, consisting of Van der Waals forces, electro-static forces, image force, meniscus forces and body forces, resist against transportation. Based on different tests with various insulating materials and thickness, 90% particle transport efficiency has been achieved.

Mixing

In a variety of processes, such as cell lyses, Polymer Chain Reaction, DNA hybridization etc, mixing of particles, cells and molecules inside micro fluidic devices determines the efficiency of the whole system. In macro scale devices, turbulence is generated and increases the contact area of the two or more fluids. Complete mixing is then achieved by molecular diffusion. Typical liquid flow in micro fluidic devices has very low Reynolds number. Molecular diffusion is responsible for the mixing in absence of turbulence and requires long time for accomplishing thorough mixing (Fig. 12). Using force perturbations to generate folds in the micro mixing device can increase the interfacial area and reducing the diffusion length for reducing the required time.

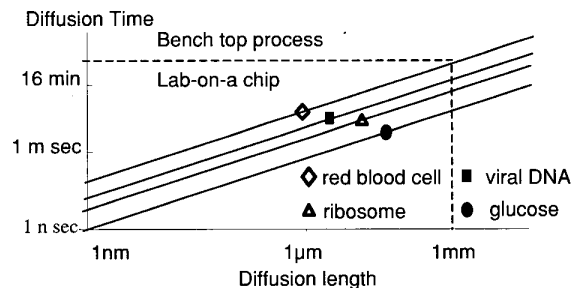


Fig. 12. Mixing time for molecular diffusion

In deterministic nonlinear dynamical systems, an unstable behavior, chaos, may happen [27]. A chaotic system is usually sensitive to its initial conditions. For example, two nearby particles with slightly different initial condition may separate far apart. The unpredictability of a chaotic flow system can serve as a practical way to improve the mixing problem. In low Reynolds number flow, the governing equations for the velocity field are linear in nature and therefore no chaos can be found. However, the equations of motion for fluid particle trajectories are not in general linear. Even rather simple unsteady 2D flow fields can result in

chaotic particle paths such as tendril-whorl flow, blinking vortex flow [28]. Stretching and folds helps in increasing contact area between species. The slow diffusion process completes the molecular mixing. If chaos is produced by properly imposed unsteadiness, the mixing among molecules is much more efficient than diffusion.

A chaos study in micro configurations has been carried out in three-dimensional serpentine microchannel [29] or in 2-D unsteady flows [30, 31]. Based on this concept, a two-stream fluid micro mixing device has been fabricated. We fabricated a micro fluidic device consisting a primary channel containing two parallel streams and several transverse channels [31]. Unsteady hydrodynamic perturbations are applied through the transverse channels for mixing the fluids in the two streams in the primary channel. When perturbations are operated at a certain frequency and amplitude range, chaos has been identified through numerical simulations. Efficient mixing has been observed in experiment. A parallel study on using DEP force to arrive chaotic mixing is also in progress. A separate paper on chaotic micro mixing will be presented in this conference [32].

Separation

In bio-analyses, particles, cells or molecules, may need to be separated from other particles. The separation process may be based on a certain physical-chemical property such as size, viscosity, diffusivity, charge, solubility and density. Electrophoresis, chromatography, ultra filtration, filtration, sedimentation, extraction, and adsorption are among the most commonly employed separation techniques. The first three techniques provide the finest purification and are more effective in micro scale.

The nature of slow mixing in micro flow is a problem for enhancing the relative motion of molecules but can be used advantageously for separation process. An interesting example is diffusion based separation device [33, 34] (Fig.13), which separates particles based on their different diffusion coefficients and also relies on the non-mixing nature in low Reynolds number fluid.

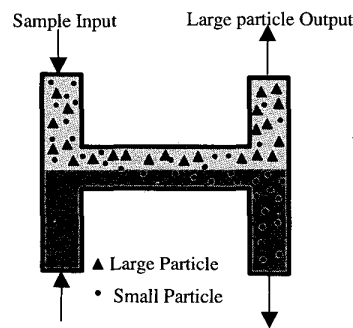


Fig 13. Diffusion-based separation

In many cases, ultra filtration is needed in biomedical applications [35]. For example, in the plasma fractionation process, the presence of even one wanted particle, such as a virus, in the filtrate stream, could be catastrophic. Ultra filtration membrane used for size-based separation can be fabricated either by solvent-casting or ion track etching process. Recently, a number

of micromachined filtration devices have been demonstrated by making either one-structural layer process [22, 36] or two-structural layer process [20, 35]. In a one-structural layer process the pores are directly made on a membrane layer and pore size is determined by photolithography. In two-structural layer process, since the nominal pore size is determined by the thickness of a sacrificial layer sandwiched between two structure layers, the pore size can be made much smaller to be about 50nm[35].

Capillary electrophoresis (CE) is widely used in biological analysis [37,38]. By applying MEMS fabrication technology, sample injector and separation capillary can be integrated in a

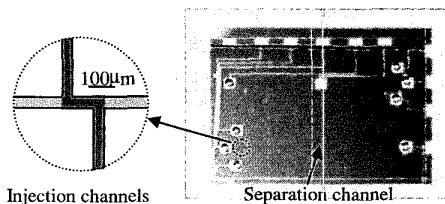


Fig 14. CE chip integrated with electrodes

simple cross-shape microchannel design (Fig.14). Both fast and efficient separation can be achieved by using short separation channels, high field strength and small volume sample plug. The efficiency of micro CE is comparable with that of the conventional CE but the analysis time is cut down to tens of seconds as compared to half an hour with the standard device [39]. By properly designing the separation channels or coupling CE with another separation technique, like chromatography, a number of two-dimensional CE chip have been reported [40, 41]; which can substantially increase the separation resolution. Recently, a capillary array electrophoresis chip was demonstrated to analyze 48 samples in a chip at the same time, which largely enhances the throughput of DNA sequencing [42].

DNA DETECTION

While conventional methods for detecting bacteria usually involve a morphological evaluation of the organisms as well as testing their ability to grow, such methods are time consuming and suffer low sensitivity and specificity. The recent development of micro systems that couple pathogen recognition with microfluidics and signal transduction has shown major advancements and potentials. A variety of biosensors, both optical and electrochemical, have been developed for the detection of pathogenic bacteria [43, 44]. In general, electrochemical methods have advantages amenable to miniaturization [43, 45, 46] and optical detection can have higher sensitivity [47].

Sensitivity and specificity are the two important parameters for biological detection. Whereas, specificity is determined by the molecular recognition scheme of each detection method, sensitivity is based on the signal transduction and noise reduction. As the device feature size scaling down to micro- and nano- meter range, it is more feasible to incorporate molecular recognition, signal transduction, and noise reduction at molecular levels under reasonable control. Also the detection time is dramatically

reduced since most biological reactions are diffusion-controlled interactions.

DNA hybridization has been intensively studied due to its highly specific molecular recognition [48]. Accurate prediction of DNA thermal denaturation/hybridization is important for several molecular biological techniques including PCR[49], sequencing by hybridization [50], antigene targeting [51], Southern blotting [52] and Biological identification [43]

Optical Detection

Evanescence wave technology is widely used in DNA hybridization based optical sensors. The evanescent wave is an electromagnetic wave, which is generated when light is completely reflected within the sensor surface. The evanescent wave penetrates beyond the optical interface into the lower reflection index medium by the distance of a wavelength and decays exponentially. Decay of the electromagnetic wave can be characterized by the penetration depth (d_p), the distance at which the intensity falls to $1/e$ of the value at the interface. Due to the limited range, evanescent wave would selectively interact with the molecules attached to or close to the sensor surface without the interference from the bulk medium. An evanescent wave fiber optic sensor, which relies on the fiber to transmit excitation light and transduces the emission signal of a fluorescence label, has been reported [53, 54, 55]. The typical concentration detection limit is about 10^{-12} M. Surface plasmon resonance (SPR) biosensor is another example based on evanescent wave. The typical detection limit is about 20 fmol mm^{-2} [56, 57].

We have developed optical genosensors based on laser induced fluorescence (LIF) system [47, 58]. By incorporating a molecular beacon (MB) probe, which is highly selective and becomes fluorescent only after hybridization with its complementary DNA, two of the major but cumbersome steps, probe immobilization and washing of gene-based biosensors were eliminated. This enables the in-flow detection technique and reduces the detection volume down to only 36 pL . In addition, by integrating a side-mirror technique, which enhances the reflectance of the sidewalls and bottom of the sensors, and the inflow sensing technique, the detection limit achieved is as low as 0.07 zmol , which is at least 3 orders of magnitude lower than many other DNA detections. Details can be found in another paper presented in this conference [47].

Electrochemical DNA Sensor

The enzyme-based electrochemical biosensor is primarily motivated by the need for a highly sensitive and selective protocol capable of rapid detection of bacteria, virus or various biological species. High specificity can be achieved by using DNA hybridization to reduce false positive and false negative signals. DNA electrochemical biosensors have been previously reported [59, 60] using graphite or carbon electrodes. Carbon-based electrodes, however, are generally not adaptable to MEMS technology when small ($< \mu\text{m}$) dimensions are needed. MEMS based three-electrode electrochemical DNA sensor has been demonstrated successfully utilizing metallic electrodes, with a

protein self-assembled monolayer (SAM) to capture the *E. coli* rRNA [61]. An enzyme is used as a biological amplifier to gain the high sensitivity without PCR. Enzymatic amplification occurs due to a high turnover number in reactions that can be detected electrochemically. The electron transfer at the electrode surface is measured amperometrically to represent the number of the enzymes immobilized by DNA hybridization through the 16S ribosomal RNA of the target cells. Therefore, the output current is proportional to the number of the target cells in the solution. At present, sensitivity in the atto ($1\text{E-}18$) mole range has been achieved.

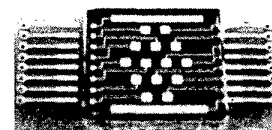


Figure 15. Micro-electrochemical DNA sensor array (14 three-electrode)

CONCLUSIONS

Concentrating, mixing, and separating particles in fluids are common processes in the industry. In bio-medical fields, the particles, DNA, proteins, cells, etc. are in nanometer and micron ranges. Micro fluidic processes have been developed to handle the particles down to molecular scales at high efficiency and greatly advanced the bio-medical diagnostic techniques.

The molecular effects affect transport properties of micro flows in a significant way. Both the molecule-wall and molecule-molecule interactions are the basic mechanisms of the observed molecular effects. Research along this direction will lead to intriguing new findings in both science and technology of the nano/micro fluid flows.

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