Visualizing Heterogeneous Flows in Micro Fluidic Devices

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Abstract

Visualization techniques for studying heterogeneous microflow in the order of hundreds of microns and for examining surface properties in the order of nanometers are devised in our laboratories. Different flow regimes and patterns obtained by varying the ratio between gas and liquid pressure are observed in a two-phase microchannel flow. For biomolecules subject to hydrodynamic and electrokinetic forces, their conformations and motions in microflows are observed by fluorescence microscopy. In addition, techniques to map the topography and the chemical properties of a solid boundary down to atomic resolution are illustrated. Atomic force microscopy is used to characterize surface atoms for resolving the topography of a bare wall and of the self-assembled monolayers deposited on a substrate. Surface slip and DNA sensor performances are determined by the surface molecular properties. Optical interferometry is used to characterize the profile of microchannels. An accurate characterization is fundamental to estimate the hydraulic resistance.

Keywords: *micro fluidics, visualization, two-phase flow, biomolecules, topography*

1. Introduction

Flows in microfluidic devices provide a fertile ground for research because of their unique features.
In the length scale region ranging from microns to nanometers the transition from continuum to molecular flow occurs. Visualization is a consolidated, powerful technique proven to facilitate the understanding of flow physics that can be extended to micro fluidic devices. However, the process can be quite challenging. For example, optical techniques are limited to sizes in the order of 500 nm because of the light wavelength. Most of the particles that flow in solution inside a micro fluidic device are smaller, yet visualization with optical methods is a tool as essential as convenient.
We implemented optical methods to examine two-phase and heterogeneous flows of biomolecules in channels with dimensions in the hundred of microns. In addition, we used atomic force microscopy (AFM) and optical interferometry to study the topographical and chemical properties of the boundary to molecular scale. These properties affect the extent of wall slip and the quality of self assembled monolayers (SAM), which is crucial to the performance of bioanalysis systems, such as DNA sensors.

2. Visualizing two-phase flow

The transport of gas bubbles in polygonal microchannels is a hydrodynamic problem with interesting practical applications in enhanced oil recovery, heat pipes, and micro methanol fuel cells. Gas bubbles can present significant problems by disturbing and eventually blocking the flow. The interaction on the boundaries between gas, liquid, and solid introduces non-linearity and instabilities in micro fluidic devices that are generally not present at such length scale.

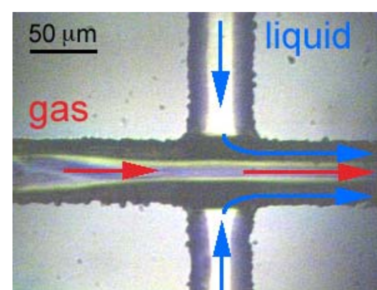

Figure 1: flow focusing to enhance capillary instability

We have studied this problem in rectangular microchannels fabricated from a silicon wafer using deep reactive ion etching (DRIE) and sealed with Pyrex glass by anodic bonding. The fluids are injected in the device trough pressurized reservoirs. The outlet is allowed to vent to the atmosphere. In the present experiments the liquid is DI water and the gas is CO_2. Flow is visualized with a CCD camera either mounted on a microscope or used with a 60 mm Nikon lens with an extension tube; in the latter case the light source is a fiber illuminator used with a semi-transparent mirror. The device, whose channels have square cross section, is composed of two sections: a 50×50 μm^2 bubble nucleation section and a 16 cm long, 200×200 μm^2 serpentine channel where the flow is investigated and the pressure drop measured. The receding contact angle $\theta = 30^0$ and the liquid fills the corners. Bubbles are nucleated using a flow focusing technique. Liquid is injected normally to the gas flow on each side of the bubble (see figure 1). The bubble breaks-up by capillary instability into an array of convected bubbles whose size and density are adjustable with the applied gas and liquid pressures.

Figure 2 displays a representative view of the identified flow patterns. The liquid pressure was kept constant at 9 psi, while and the gas pressure ranges from 3 to 15 psi. For low gas pressure, bubbly flow is characterized by bubbles smaller in diameter than the channel width a. As the gas pressure increases, the bubble volume V increases. For partial wetting, when the contact angle $0^\circ < \theta < 45^\circ$, and when $V > a^3$, bubbles touch the walls and liquid fills the wedge [1]. A contact line between gas, liquid and solid is created as can be seen for the wedging flow figure 2b. With our parameters, the typical velocity for the bubbles is in the order of cm/s.

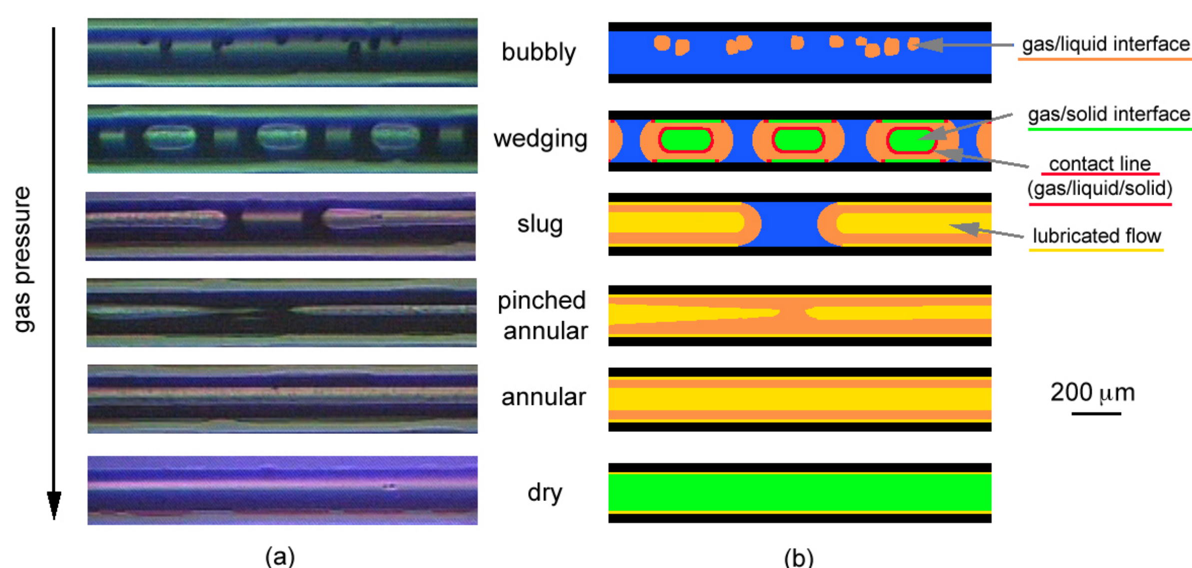

Figure 2. (a) flow patterns, (b) interfaces

As the void fraction increases, the flow becomes unstable and perturbations are amplified. The transition from wedging to a slug flow presents hysteresis. The slug flow is characterized by large bubbles with $V \gg a^3$ and separated by a small amount of liquid. There is a thin film of liquid between the bubbles and the walls. The bubbles velocity is in the order of m/s and the capillary number Ca > 10^{-1}. For such Ca, the receding contact angle is zero and there is no contact line. As the gas pressure is increased, the flow becomes annular, composed of a single large bubble. For annular flows at low pressure, a part of the liquid travels along the walls, while at higher pressure the liquid is strictly confined in the corner of the channel. Finally, for very high gas pressure, the gas flow dries the liquid in the corners.

Flow visualization is essential to identify and quantify flow patterns, void fraction, contact angles, bubbles shape, and velocity. Combined with pressure drop measurements, visualization allows an accurate description of the important hydrodynamic aspects of two-phase flow in microchannels.

3. Visualizing biomolecules

Visualization of biomolecules in micro and nano fluidic devices often leads toward new discoveries. Direct observation of the biomolecules down to single molecule level can provide invaluable insights into biological science and technology [2]. In advancing bio-nano technologies, visualization of bio-molecules in a flow becomes an interesting task for many experimentalists.

For observing subjects by visible light, the smallest size is limited in the order of 500 nm, which is the range of light wavelength. The double helical structure of DNA has diameter of 2 nm. Its length varies in a wide range, from nanometers to meters for human DNA, but the radius of gyration of small DNA molecules is only in the range of nanometers. Most proteins also have length scales of nanometers, as well. Direct measurements need to be made by atomic force microscope or other instruments with angstrom resolutions. On the other hand, we still can study the dynamics of these nanoscale biomolecules by visualizing them in a micro fluidic device through attached fluorescent molecules.

We have performed experimental studies to investigate the dynamics of DNA molecules in microchannel flow. T2 DNA (164kbp) was stained with YOYO-1 (Molecular Probes, Inc) at a dye to base-pair ratio of 1:5. The DNA-dye complex was allowed to equilibrate for more than an hour before the experiment. The micro fluidic devices applicable to direct molecular visualization were fabricated on 4-inch double-sided polished silicon wafer by micromachining. Bulk etching, such as KOH or deep reactive ion etching, was used to fabricate channels with different geometry, depending of the required experimental condition. The channels were sealed by anodic bonding with Pyrex glasses to provide an optical window for direct visualization. The micro fluidic chips were then loaded on an epi-fluorescence microscope (Olympic IX70) for DNA dynamics study. Intensified CCD cameras (Hamamatsu C5909, Videoscope ICCD-350F) were used to pick up the low intensity light emitted from the DNA molecules. The time-dependent conformation of DNA could then be obtained from the video information (Fig. 3).

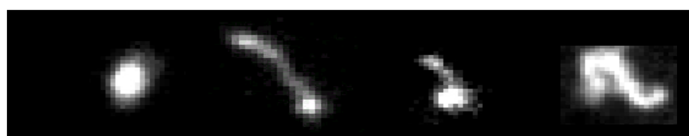

Fig. 3. Time-dependent conformation of DNA molecules

Technical challenges may affect the observation of the molecular dynamics of biomolecules. For example, photobleaching effect and light-induced DNA cleavage are observed under high intensity illumination, which is necessary for observing molecular dynamics at

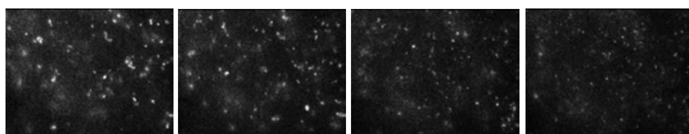

Fig. 4. Photobleaching of λ phage DNA molecules stained with YoYo-1. Each picture is separated by 5 sec. The width of the pictures is 123 μm.

single molecule level [3]. Photobleaching is the decay of fluorescence intensity due to the photochemical reaction of the dye molecule. Figure 4 illustrates the decay of the fluorescence intensity of λ-phage DNA molecules under illumination. To reduce this effect, we enzymatically removed oxygen and free-radicals by adding 50 mg/ml glucose oxidase (Sigma), <2 mg/ml catalase (Sigma), 4% beta-mercaptoethanol (Fisher), and ~10% (w/w) glucose in the buffer. Fig. 5 shows the reduction of the photobleaching effect under different concentrations of glucose oxidase. We could observe the dynamic of the DNA molecules for more than 20 minutes under our improved experimental condition.

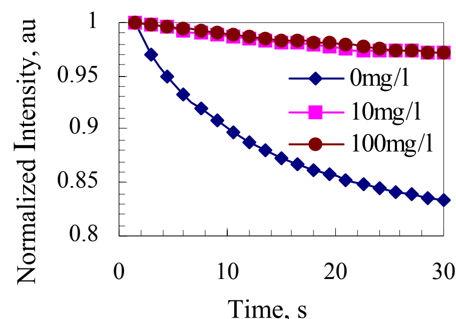

Fig. 5. Reduction of photobleaching effect for different concentrations of glucose oxidase.

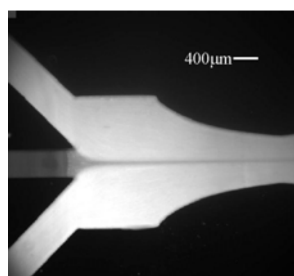

Fig. 6. Channel for hydrodynamic focusing of DNA molecules.

We also studied the conformational dynamics, such as deformation and orientation, of isolated T2 DNA molecules (164kbp) subject to hydrodynamic focusing. A micro channel with three inlets (figure 6) was designed and fabricated for this study. Buffer and DNA solutions were delivered into the microchannel with two digital syringe pumps. Buffer solution was pumped into the side channels with a T-shape flow divider, while DNA solution was pumped into the middle channel. The channels with hydrodynamic focusing were able to generate a hydrodynamic force sufficiently large to deform the DNA molecule far from the random coil equilibrium conformation.

Due to the small transverse dimension, in a simple rectangular microchannel the shear force is sufficient to deform DNA molecules. Figure 7 shows some conformational changes of DNA molecules under steady shear. In addition to the stretching (fig. 7a) and coiling (fig. 7b) of the molecules, we also observed other transitions of molecular conformation, such as folding (fig. 7c) and mass redistribution (fig. 7d). When the flow was suddenly stopped, a stretched DNA molecule would coil back. We could observe this relaxation process with detailed conformational information for single DNA molecules. The relaxation time was governed by the force balance between the DNA elastic force and the hydrodynamic drag, and could be directly measured with video fluorescence microscopy [4].

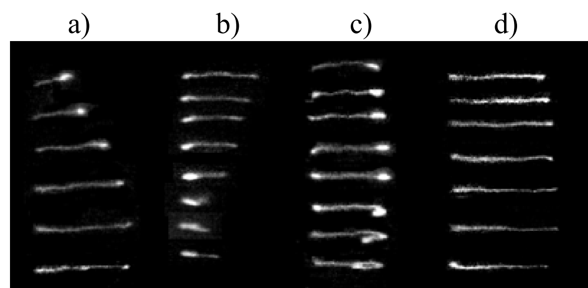

Fig. 7. Video fluorescence images of conformation changes under steady shear in a rectangular microchannel. The scale bar represents 10μm.

In another experiment, we studied the behavior of biomolecules under the influence of electrokinetic forces. These forces, generally very effective in micro fluidic device, can be applied to micro total analysis systems for different fluidic operations, such as mixing, separation, and concentration [5]. Visualization techniques provide the tools to characterize different electrokinetic force fields and their interaction with biomolecules. To understand the response of biomolecules subject to an external electric field, λ-phage DNA digests (3530, 4878, 5643, 5806, 7421, and 21226 bp) and T2 DNA molecules were labeled with YoYo-1 for direct visualization. The dynamics is directly observed with fluorescence microscopy as discussed earlier. Microelectrodes with different shapes and layouts were designed and fabricated. For better metal coverage, microchannels with smooth and tapered sidewalls were etched with KOH from silicon substrates. After thermal silicon oxide was grown for electrical isolation, Cr and Au layers were deposited by e-beam evaporation and patterned by lift-off. Numerical simulation tools (CFD Research Corporation) were used to visualize the electric field distribution for optimization of the electrode design. Fig. 8 shows a comparison of two different designs. The 3D

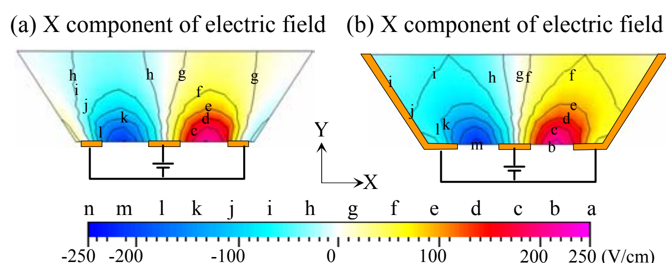

Figure 8. Simulation of electric fields generated by two electrode designs.

electrode design consisting of a middle electrode and two electrodes covering the sidewalls allows wider and denser electric field for molecular manipulation (fig. 8b).

Electrokinetic manipulation was applied to improve the detection efficiency of laser induced fluorescence (LIF) system based single molecule sensing [6]. In LIF based detection it is desirable to have a small probe volume (smaller than 100fL) to minimize the background radiation caused by Rayleigh and Raman scattering of the laser beam by solvents. On the other hand, because of the small probe volume, the LIF based detection has a potential problem with low detection efficiency. The efficiency of conventional on-column detection with capillary can be smaller than 1%. We improved the detection efficiency by developing an electro-molecular focusing technique to concentrate the sample into the probe region (Fig. 9).

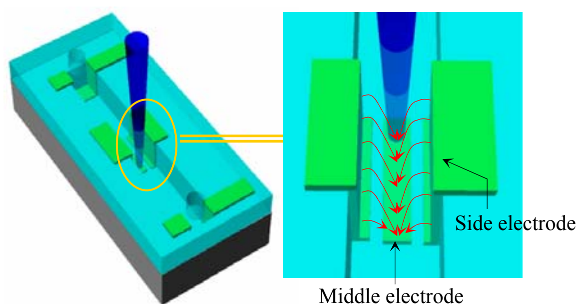

Figure 9. Conceptual schematic of 3-D electro molecular focusing. Positive potential is applied to the middle electrode while both side electrodes are grounded. Negatively charged DNA molecules are concentrated towards the middle electrode due to the electric field (red lines).

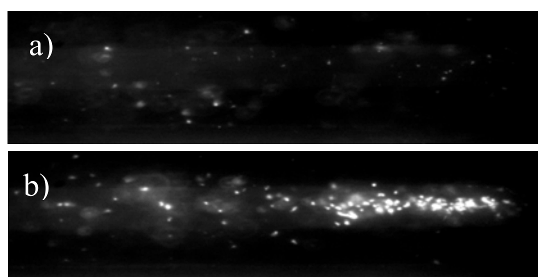

Fig. 10. Focusing of DNA molecules: a) before focusing; b) after focusing.

Figure 10 shows electrokinetic focusing of T2 DNA molecules under continuous channel flow condition. As shown in fig. 10b, the DNA molecules were focused within a region only ~3µm wide. The detection efficiency can be greatly enhanced if the LIF detection is performed on the downstream end of the focusing electrode.

4. Visualizing Surfaces

In micro fluidic devices, the study of flow phenomena is more effective if accompanied by the detailed visualization of the bounding surfaces. Due to the small transverse dimensions, the interaction between fluid and boundary can express effects adverted across the entire flow field. Several recent studies [7-10] have shown that wall roughness in the nanoscale range and surface chemistry regulate the slip of the molecules of a liquid in contact with a solid surface. In particular, the extent of wall slip is directly dependent on the smoothness of the surface: the greatest degree of slip is reached on atomically smooth surfaces, such as bare mica and sapphire, and slip disappears when the rms roughness is around several nanometers. Since slip lengths as large as 1 micron have been determined, this phenomenon needs to be considered in micro and nano channels. It is worth noting that a boundary with this level of roughness is traditionally considered smooth. Novel

techniques are employed to measure the roughness of the wall and visualize its surface with unprecedented accuracy.

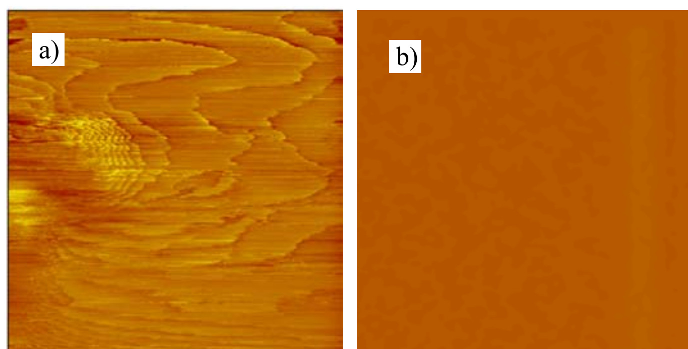

Figure 11. AFM scan of a mica surface. a) The scan area is 100x100 microns. Several cleaving planes are visible. b) 500×500 nm^2 detail. The scan shows atomic smoothness.

Atomic force microscopy (AFM) can image a flat substrate to atomic level (fig. 11). In this technique a sharp tip mounted on a cantilever beam scans a surface. The deflection of the beam, which is generally measured optically, relays information on the surface properties. The limit to the resolution is imposed by the geometrical size of the stylus probe. Atomic resolution is reached nowadays by using atomically sharp tips manufactured from silicon with MEMS processes. Carbon nanotubes have also been attached to the probe to improve the quality of the scans.

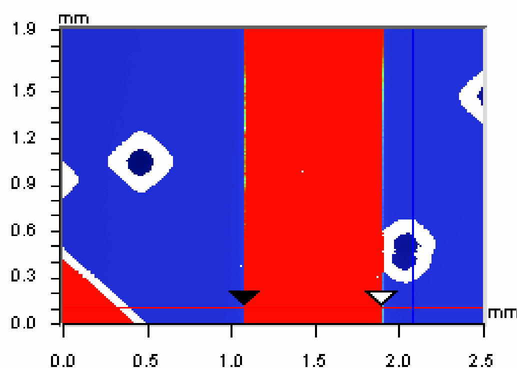

Figure 12. Detail of a micro fluidic device (VSI scan). Several isles with the characteristic pyramidal shape left unetched during the KOH process are recognizable on the bottom of two channels.

There are instances where the topology needs to be visualized on wider areas and less than atomic resolution is necessary. The same way that wall slip depends on the surface roughness, a channel's hydraulic resistance depends on its geometry. Depth and width of a channel in a micro fluidic device are in the order of 100 microns with length on the order of millimeters. Ideally the cross section is rectangular or trapezoidal; however, it can sensibly depart from the ideal shape because of the waviness of the surfaces, typically on the order of microns. In addition, the manufacturing process can introduce deviations from the expected geometry and non-uniformities in shape of around several microns. In same cases, isles of a few tens of microns or even larger are left un-etched on the bottom of the channel (figure 12). These factors represent a serious problem, since the hydraulic resistance of a channel is sensitive to its dimensions, particularly to its depth, because of the third power dependence. Therefore, the surfaces of the channel must be thoroughly visualized and characterized prior to the final packaging step. For this purpose, we have used a WYCO optical interferometer, whose techniques are optimized to map the topography of micromachined devices. Different modalities are available depending on the type of measurement needed. For example, to measure the depth of a trench or of a channel etched on a substrate, vertical scanning interferometry (VSI) can be used. The technique is based on the analysis of the interference fringe pattern originating from the combination of two light beams, one reflected from a smooth reference surface, and one reflected from the sample. As the objective is moved perpendicularly to the surface to scan it at varying heights, the interference pattern at each

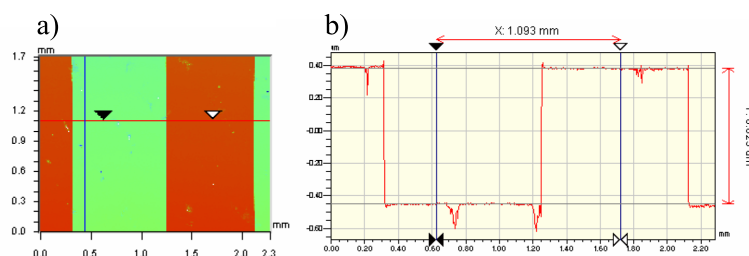

Figure 13. Detail of an array of submicron deep channels etched on glass (VSI scan). a) Top view. b) Cross section

height is acquired and stored. Finally, the different scans are internally processed and a 3-D mapping of the surface is produced. In general, various internal functions are available to perform several operations, such as calculating the mean depth in a given area, or visualizing the cross section of the sample at an arbitrary location. The vertical range of VSI can be larger than the thickness of a standard silicon wafer, so that etched channels of any depth can be scanned. Moreover, a vertical resolution in the nanometer range and an imaging area as large as ¼ squared millimeters allow a rapid and accurate characterization of typical channels in micro fluidic devices (Figure 13).

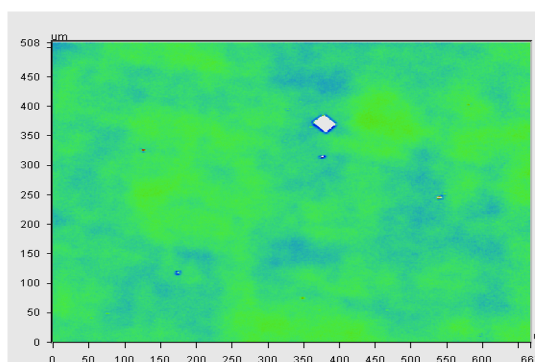

Figure 14. PSI scan of a 500x650 μm^2 area of the bottom of a smooth silicon channel. A defect affecting the otherwise nanometer level roughness is clearly visible.

A second mode, called phase-shifting interferometry (PSI), offers an angstrom scale vertical resolution. Differently from VSI, the reference surface is moved of a small, preset amount to cause a phase shift between the two beams of filtered light. The intensity of the interference patterns is recorded at different phase shifts and eventually converted to wavefront data by integration. This second mode, suitable to visualize smooth substrates, cannot compete with the AFM technique in terms of resolution, but carries the advantage of much a larger imaging area, which is preferable when inspecting a surface for non-uniformities or defects (figure 14).

In addition to surface roughness, also the chemical properties of a surface have an effect on wall slip. In particular, the lower the interaction between fluid and boundary, the greater the extent of slip. The slip of water on a surface increases with the degree of hydrophobicity of the surface. The chemical properties of a microchannel's surface can be conveniently controlled by means of chemical treatments, generally by deposition of a self-assembled monolayer (SAM). SAMs are composed of linear molecules that spontaneously absorb to a specific substrate and arrange among themselves in an ordered, tightly-packed fashion. The absorption of the constituents occurs though the functional group at one end of these molecules, while the functional group at the other end confers the chemical properties typical of the monolayer. Several layers of different SAMs can be grown on top of each other. These coatings are normally used to turn a surface from hydrophilic to hydrophobic, and vice versa, with broader effects than simply affecting the slip of the fluid. SAMs of different kinds can be employed for multiple purposes in micro fluidic devices, for example to avoid fouling of the microchannels, to extract molecules from the liquid medium, or to provide a receptive surface for the immobilization of the bioassay on the detector's electrode of a DNA sensor [11]. In the last case, good coverage is essential to reduce the signal noise and increase the sensitivity of the detector. SAMs can also be used to alter the behavior of a flow field, such as in the case of two-phase flow, because of they change the contact angle with fluid. In a novel application [12, 13], the fluidic pattern of a micro device can be reconfigured at will by reversibly changing the surface chemistry of its microchannels through the use of SAMs. A uniform surface coverage is essential for the effectiveness of a monolayer. One method to estimate the quality of the treatment is by measuring the contact angle between a droplet of a certain liquid (generally water) and the surface (figure 15). The contact angle is affected by the free energy of the surface, which in turn depends on the relative area of substrate covered by the monolayer. This method can offer only indirect evidence of the quality of the SAM. To assess the local uniformity of the coverage, the monolayer must be characterized in detail. AFM techniques are suitable to image an SAM and estimate the quality of the monolayer (Figure 16).

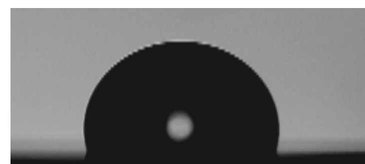

Figure 15. Contact angle measurement on a silicon wafer surface treated with an SAM of octadecanethiol.

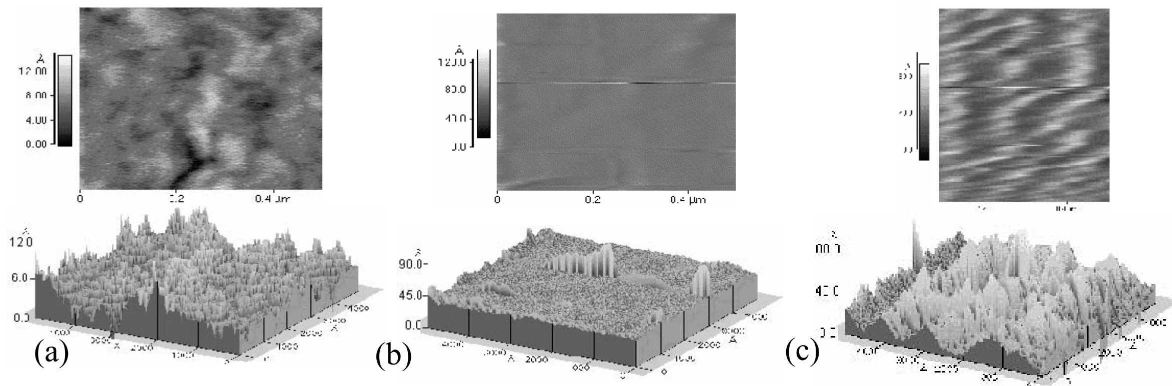

Figure 16. AFM of a streptavidin SAM formation on Au. (a) bare Au only; (b) full coverage with protein dragging; (c) partial coverage with protein islands [11].

5. Conclusions

We have illustrated optical techniques that we have devised for the study of heterogeneous flow in micro fluidic devices. In addition, we have discussed the application of AFM and optical interferometry for the visualization and characterization of solid boundaries.
Through visualization, we have been able to study two-phase flow and the dynamics of biomolecules, such as DNA, in microflows in the order of hundreds of microns. In micro two-phase flow, the visualized flow patterns depend on the capillary number, which is adjusted by changing the supply pressure ratio between the liquid and the gaseous phase, and on the interaction energy between the boundary and the flow. In the inhomogeneous flow of DNA molecules in buffer solution, we have achieved single molecule visualization by fluorescence microscopy. DNA molecules subject to the hydrodynamic force fields in micro fluidic devices undergo conformational changes such as stretching, coiling, folding, and mass redistribution. Moreover, electrokinetic force fields, created by properly designed electrodes embedded in the micro device, are used for molecular focusing of the macromolecules to enhance the detection efficiency.
Finally, we have studied the topography and chemical properties of the boundary and of deposited SAMs for the role they play in controlling the amount of wall slip of the flow and on the performance of biosensors. Uniformity, smoothness, and compactness of the SAMs deposited on the electrodes of biosensors, which can be visualized by means of AFM techniques, is required to enable detection with high sensitivity.

Acknowledgments

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