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Microfluidics and Chromatography with an Atomic Force Microscope

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A combined atomic force microscope (AFM) and Raman spectrometer is presented as a microfluidic device for pumping, sampling, and trace chemical analysis. The AFM tip−cantilever provides a mechanism for shear-driven pumping of fluids in microchannels. Shear-driven pumping allows rapid flow rates and avoids the limitations of conventional pumping. The AFM's ability to translate sub-femtoliter volumes of fluid also proves a mechanism for fluidic switching and sample injection. In addition, the AFM is used to image liquid surfaces in microchannels and remove samples for very sensitive spectral analysis. Surface-enhanced Raman spectroscopy localized near the AFM tip provides chemical information of the sampled fluids. The results demonstrate the feasibility of integrating the AFM with microfluidic circuits and shear-driven chromatography and the potential for nanometer-scale chromatography.

Scanning probe microscopes (SPMs) and the associated technology are now established in microscopy and nanotechnology. The most widely utilized of the probe microscopes is the atomic force microscope (AFM). With an AFM, a tip mounted on a microfabricated cantilever is scanned over the substrate and the interaction between the tip and the substrate is detected by monitoring the deflection of the cantilever.[1−3] It was quickly realized that by exploiting the properties of a sharp tip in proximity to the surface, the technology could be used to provide localized spectroscopic, chemical, and physical property information.[4−6] In addition, the probes could be used manipulate and engineer surfaces on the nanometer scale. This is achieved through local heating, optical etching, and deposition of material from the AFM tip to create nanometer-scale structures.[7−9] The addressable modification of surfaces using probe microscope developed techniques is now being exploited in recently developed memory devices.[10] SPM technology has emerged as fundamental to the study of nanometer-scale phenomena and devices. Similarly, microfluidic devices are finding widespread scientific and industrial applications. Microfluidic devices rely on a variety of geometries and physical phenomena and exploit new variations of well-known fluid dynamics effects for chemical analysis, synthesis, and processing.[11]

The work presented here demonstrates microfluidic applications of a combined atomic force microscope and Raman spectrometer (RAFM) for nanometer-scale surface sampling, trace spectrochemical analysis, and microfluid pumping. It has been previously demonstrated that an RAFM probe tip can be used to selectively remove surface layers for subsequent microchemical analysis using infrared and Raman spectroscopy.[12,13] This study demonstrates the feasibility of using an integrated RAFM for addressable separation and analysis in microfluidic devices. The RAFM was used to image liquids in open microchannels and subsequently remove small volumes of liquid material from open microchannels in a capillary-like process on to an AFM tip and cantilever. This is the reverse of the so-called dip-pen nanolithography process, where materials are deposited on a surface from an AFM tip.[14] Once the material is collected on to the RAFM's special cantilever-tip, the local surface enhanced Raman spectrum (SERS) is acquired with very high sensitivity.[15]

During the course of this work, the AFM tip−cantilever was found to be an effective device for producing shear-induced flows in open microchannels and for transferring fluid between channels. Shear-induced flows result from axially sliding the AFM tip−cantilever over a liquid-filled open channel. The viscous drag establishes a net flow with a linear velocity gradient. This is a smaller, more localized implementation of the fluid pumping used in shear-driven chromatography (SDC) instruments.[16] Shear-driven pumping has the advantage of providing rapid flow rates through microchannels without the pressure drop limitation of conventional pressure-driven pumping. Electrically driven pumping systems, as used in capillary electrophoresis, have a similar

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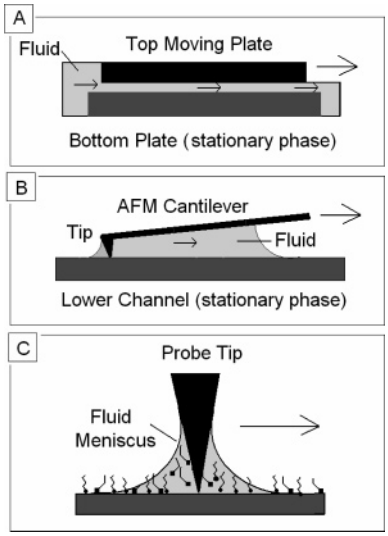

Figure 1. (A) Cross section of the basic shear-driven microfluidic pump. The liquid in a channel is pumped using the shear force of the top moving plate. The viscous drag establishes a net flow with a linear velocity gradient. The bottom plate may be functionalized to provide chromatographic separation. Panel B illustrates the use of an AFM cantilever functioning as the microfluidic shear plate. (C) shows a nanometer-scale implementation where the AFM tip provides highly localized shear translation at a tip-localized meniscus. The AFM cantilever or the AFM tip provide a microfabricated structure that allows precise manipulation, switching, and shear pumping of fluids.

pumping limitation imposed by the Joule heating effect that places an upper limit on the applied voltage gradient.[17] Figure 1 compares the original shear-driven pumping concept and the AFM implementation of shear-driven flow.

The demand for rapid, miniaturized analytical separations requires performance improvements over the conventional pressure and electrically driven pumping systems. SDC has been theoretically evaluated and demonstrated as a mechanism for performing rapid separations.[17,18] One of the limitations of SDC and other forms of miniaturized chromatography is in sample detection. As demonstrated in this study, AFM-tip-localized SERS is a potential detector. The AFM itself, with its ability to image individual atoms, is a remarkably sensitive detector that can be used to analyzed separated components. The feasibility of integrating RAFM and SDC is established in this work with AFM-based mechanisms providing switching, injection, pumping, and detection.

EXPERIMENTAL SECTION

The Raman−Atomic Force Microscope. The integrated Raman and atomic force microscope (RAFM) instrument used in this work was developed to provide simultaneous topographic AFM imaging and highly localized Raman spectroscopy by exploiting the SERS effect at a specially coated AFM tip. A detailed instrument description is given elsewhere.[15] Figure 2 shows a labeled photograph of the instrument. The RAFM is the integration of a commercial AFM and Raman systems. The AFM module uses a Digital Instruments (Santa Barbara, CA) Nanoscope 4 controller with a modified D3000 head and custom-built stage.

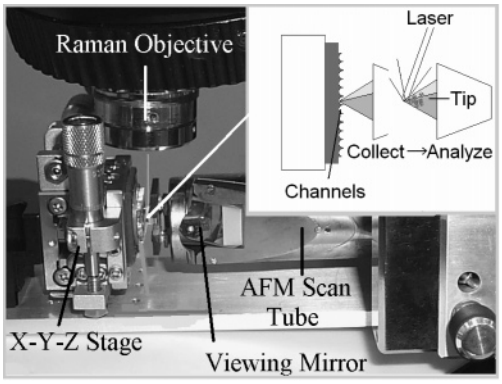

Figure 2. Labeled photograph of the RAFM used for microfluidic applications. The AFM tip provides an addressable collection of fluids and analytes. The tip and cantilever are coated with gold, which provides for localized surface enhanced Raman spectroscopy for very sensitive detection and spectrochemical information. The insert schematic shows a cross section of the channel substrate that may be imaged and sampled with the RAFM tip for surface enhanced Raman analysis.

The Raman microprobe system is a Kaiser Holoprobe (Kaiser Optical Instrument Systems, Ann Arbor, MI) attached to an optical microscope with a modified microscope stage to accommodate the AFM head. A laser excitation wavelength of 785 nm is used in this work. The Raman beam size is approximately 5 μm and the power is adjustable up to 10 mW. The system is also equipped with dual optical microscope viewing.

Materials and Chemicals. Silicon tapping mode AFM tips (type OTESPA7) were used from Digital Instruments (Santa Barbara, CA). The SERS-active AFM tips were fabricated using Novascan (Ames, IA) alumina-coated tapping mode tips. These tips were gold-coated using plasma sputtering to provide SERS activity.[15] The test liquids were ricinoleic acid (Acros) and 1-decanol (Aldrich) that was microsyringed on a surface with open microchannels. The open microchannels used in these experiments were gold, with a grove width of approximately 950 nm and 125-nm depth. This was fabricated from nitric acid etching of a gold 3M Brand compact disk, which provides a very clean gold surface with ruling suitable as microchannels.[19]

RESULTS AND DISCUSSION

AFM Fluid Imaging in Microchannels. The ability of the AFM to image fluids in a microchannel array was first examined. This is necessary if it is to be incorporated in fluidic devices. It was not previously known what the effect of a liquid in the microchannel would have on the AFM scanning process. To avoid evaporation effects, low-volatility ricinoleic acid or decanol was used as test fluid. Higher volatility liquids could be used if sealed in a closed system in vapor−liquid equilibrium. The channels were filled by microsyringing a drop of liquid on a gold channel array substrate. Liquid slowly migrated down the approximately 1-μm-wide, open channels. The channels before and after filling were imaged with the AFM using tapping mode (Figure 3). The microchannel stabilized the liquid, and the tapping-mode AFM imaging minimally disrupted the liquid surface. The AFM phase contrast image, that is very sensitive to material surface variation,

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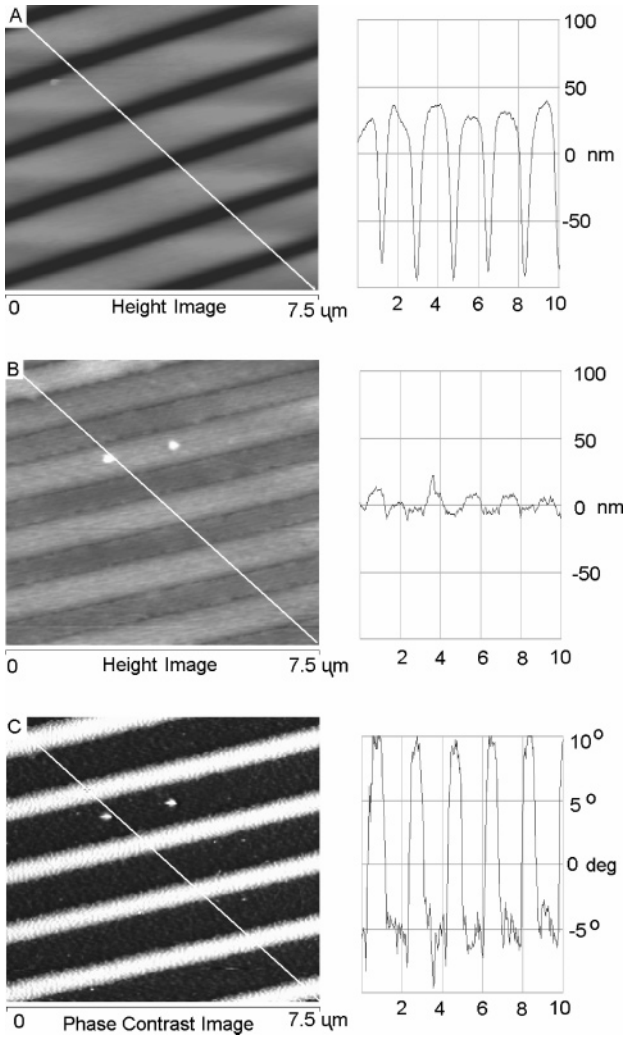

Figure 3. (A) Tapping-mode AFM image and cross section of an empty channel array. (B) An array of ricinoleic acid filled microchannels revealing the liquid contained in the gold-coated channel. The microchannels provide stability to fluid that allows the fluid surface to be imaged by AFM. (C) AFM phase-contrast mode of the filled channels showing that the liquid is effectively contained in the channels.

reveals that the liquid is completely contained in the channel (Figure 3C). The open microchannels provide effective stabilization of the liquid for tapping-mode AFM imaging. The imaging of liquid surfaces confined in microchannels may have other applications, such as studying fluid−surface interactions.

Trace Sampling and Raman Analysis. Next it is demonstrated that the RAFM can be used to remove fluid from a microchannel for trace analysis. To remove the liquid on to the tip−cantilever the AFM is operated in contact mode. The AFM tip is placed on a fixed point on the liquid at the center of the channel by zooming in on the desired location for 1−5 s. This allows the fluid to migrate up the AFM tip−cantilever. The tip is then disengaged from the surface and the change in resonance frequency is noted to determine if material was collected. While disengaged above the sample, the surface-enhanced Raman scattering of the tip-separated material could be measured in the RAFM system. Figure 4 shows the surface-enhanced Raman spectrum of ricinoleic acid on the AFM cantilever approximately 1 μm behind the AFM tip. The amount of material collected on

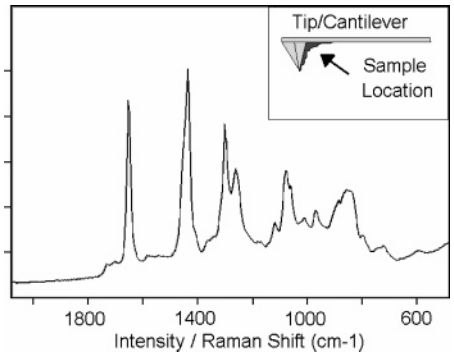

Figure 4. The small quantities of liquid riconoleic acid separated from the microchannel may be analyzed at the RAFM tip or on the cantilever structure near the tip. Shown here is the surface-enhanced Raman spectrum of the collected material on the cantilever. This is a low-femtogram quantity of collected material. Note that surface-enhanced Raman scattering methods are sensitive to single molecules, depending on the molecular functional groups.

to the AFM tip or cantilever depends on how long the tip is in contact with the fluid channel. If very small (sub-femtogram) amounts of material are removed, the SERS spectra are acquired from near the RAFM tip apex. This geometry with side illumination of the RAFM probe tip by the Raman excitation laser provides the greatest SERS sensitivity.[15] If larger amounts of fluid (picograms) are collected, then the sample-coated cantilever arm surface (also SERS active) could be used as a substrate to acquire the spectra. This is achieved by changing the angle of incidence of the Raman laser such that it is normal to the AFM cantilever bottom surface. The larger quantities collected on the cantilever arm could be analyzed by conventional Raman or infrared spectroscopy. It has been previously demonstrated that microanalysis of residue collected on the cantilever may also be achieved using Fourier transform infrared spectroscopy.[12]

The SERS technique is extremely sensitive, and when coupled with the localized sampling afforded by the AFM tip, it could be a selective detector for microfluidics and chromatography systems. When a solvent with a dissolved analyte is sampled, the solvent could be evaporated before spectral analysis of the analyte. The ultimate sensitivity of the SERS measurement depends on the molecular species and if it is excited at the molecules electronic resonance frequencies. SERS has demonstrated sensitivity for single molecules under optimal conditions.[20,21] AFM probes may be functionalized to modify the affinity of the tip to the collected fractions.[22] This could add chemical separation to the AFM sampling operation.

Quantitative Analysis Using Cantilever Resonance. It is difficult to quantitatively measure trace amounts of collected material by SERS. However, it is worth noting that resonating cantilevers can be used for very sensitive mass measurements.[23] The collected mass, Δm, can be calculated from the shift in the cantilever resonance frequency from f_0 to f_1 by the equation $1/f_1^2 - 1/f_0^2 = \Delta m/(4\pi^2 K)$ and K is the cantilever spring constant.

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Interestingly, the sensitivity for cantilevers with resonance frequencies approaching 1 MHz have calculated sensitivities down to 10^{-21} g, that is, at the single molecule level.[24] While cantilevers optimal for the most sensitive quantitative analysis are not optimal for AFM imaging, AFM cantilevers resonating in the 20−200 kHz may have better sensitivity than quartz crystal microbalances or surface acoustic wave devices that operate in the 5−500 MHz ranges.[24]

Shear-Pumping Fluids with an AFM. Next, the ability of the AFM to pump liquid in microchannels with the cantilever and tip is demonstrated. As noted above, the AFM when operated in contact mode can remove fluid from the microchannel. The fluid flow from the microchannel to the cantilever can be observed using the AFM systems optical microscope. Once the wicking stopped, the AFM tip would trap a microdroplet between the cantilever and surface around the tip perimeter.[25] The amount of fluid trapped by the tip−cantilever could be varied depending of the film thickness of the sampled reservoir. The optical microscope observed drops ranged in size from approximately 5 pL to less than 0.03 pL. The trapped fluid could be translated to the full AFM scan range of 120 μm at a rate of 1000 μm/s. Figure 5A shows an optical micrograph of a micrometer-scale drop trapped at the AFM tip. The gap where the fluid resides under the cantilever is determined by the tip height (~4 μm) and to a lesser extent the contact force. These are both controlled AFM parameters.

The ability to drive the flow of fluid in a single open microchannel was accomplished by sampling the fluid from a reservoir droplet placed on the microchannel array surface. The AFM parameters were set so the tip does not retrace after scanning down the channel. Figure 5B shows the AFM pumping liquid along the microchannels that were sampled from a reservoir. As with the larger droplets, the trapped fluid could be pumped along to the full AFM scan range of 120 μm. It should be noted that an AFM tip can deposit fluid to the surface using the well-known dip pen nanolithography (DPN) process.[14] However, The DPN process typically involves smaller amounts of material deposited at less than 100 nm line widths. The observed shear translation of fluid was determined not to be simple deposition by DPN under these conditions. This was confirmed by lifting the AFM tip away from the reservoir and placing on an empty microchannel without significant deposition being observed under these operating conditions. Fluid from one channel could be translated across to a parallel channel through successive passes from one channel to another, as shown in Figure 5C. The injected fluid length is ~3 μm long with ~0.1 fL of fluid. This provides a very sharp injection mechanism to the head of an open microchannel column.

The use of an AFM tip−cantilever structure for shear-driven pumping is new and may have useful applications. Desmet et al. has provided the theoretical and practical demonstration of using shear-driven chromatography with a linear translation distance for separation on the order of 1000 μm.[16,19] This is larger than usual AFM scan size but could be achieved using a liner translator on a large capacity AFM stage. As noted by Desmet and Clicq, shear-driven fluids can be pumped at high fluid velocities in small channels without the high pressures of conventional pressure-driven liquid pumps. Shear-driven chromatography is compatible with the common high-pressure chromatography modes such as reverse phase or size exclusion separation. The shear-driven pumping also has advantages over electrically driven liquid pumping (used in capillary electrophoresis related methods) which is bound by voltage drop limitations that are the result the Joule heating effect.[17] As demonstrated here, the AFM tip−cantilever may serve as the shear plate for micrometer-scale channels. Sample injection may be achieved through manipulation of the sample with the tip and "injecting" into a mobile-phase-filled channel. In addition, the AFM may be a useful tool for directly measuring small-scale fluid forces by exploiting the AFM's ability to measure lateral or torsion forces on the tip−cantilever.[3]

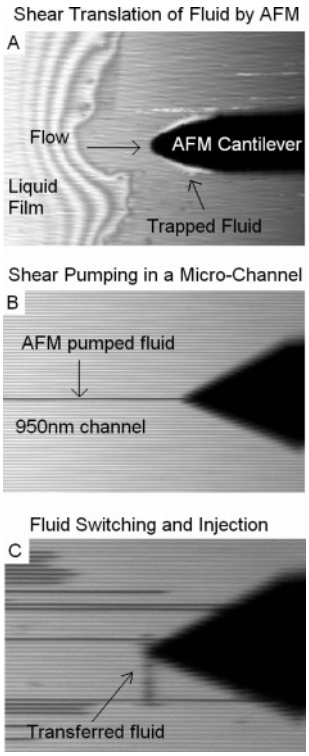

Figure 5. AFM's ability to pump and manipulate liquid illustrated by these optical microscope images. Panel A shows a ~10 pL droplet trapped between the surface and cantilever. The fluid is visible around the end of the cantilever. This fluid was translated from the edge of a decanol film reservoir. Panel B shows fluid in a ~950-nm-wide channel that was pumped by the AFM. The dark line is the fluid-filled channel. The fluid can be rapidly translated 120 μm, that is, the maximum scan size of the AFM. Panel C demonstrates the ability of the AFM to inject approximately 0.1 fL of fluid from one channel to into the empty channels. This provides a mechanism of injecting and switching fluids. Note, for scale, the width of the AFM cantilever is 50 μm.

FUTURE WORK AND CONCLUSION

This work establishes the feasibility of integrating AFM to microfluidics and chromatography. Future work would be to explore chromatographic separation and detection using the AFM and tip-localized spectroscopic detection techniques. Shear-driven chromatography has been demonstrated with approximately 1000 μm separation lengths and rapid separation times of less than 0.5

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(25) The fluid wicking could be controlled by modifying the AFM tip and cantilever surface chemistry. "A hydrophobic coating, using a perfluoronated polyether, was found to inhibit the wicking of water on to the cantilever".

s.[26] In order for an AFM-based shear-driven chromatography system to reproduce these results, the AFM would need to be modified to scan at longer (1000−2000 μm) lengths with accurate linear tracking in an open column.

The potential for the AFM to provide nanometer-scale shear-driven flows will be considered. Liquid trapped at an AFM tip can be highly localized at the molecular scale. This has been noted for the artifacts produced in imaging surfaces with molecular hydration layers. Exploiting this AFM-tip-localized water meniscus was part of the concept motivating the development of dip pen nanolithography.[9] Similarly, a tip-localized meniscus may serve as a chromatography "eluent" with the surface as the stationary phase. In this case there could be an equilibrium established between analytes absorbed on the surface and the tip−meniscus fluid. The partitioning of analytes during translation could be exploited for chromatographic separation on the nanometer scale. More speculatively, separation may be achieved on an atomic scale by partitioning of atoms or molecules from a surface to a functionalized AFM or scanning tunneling microscope (STM) tip. Applying an electric potential between the tip and surface could be used to control partitioning between the tip and surface. The feasibility of the atomic scale translation of atoms partitioned between the tip and the surfaces is supported by the STM experiments where the dynamic transfer of atoms using STM tips has been analyzed down to the atomic scale for use in atom manipulation.[27] The lateral transfer of atoms has been addressed by Stroscio and Celotta.[28] Such atomic scale separations would be better described by quantum mechanics than conventional chromatography theory.

In conclusion, it has been demonstrated that an AFM can be used (a) to image liquids in microchannels, (b) to separate fractions from channels for very sensitive spectrochemical analysis, (c) to perform sensitive gravimetric measurements, (d) to switch fluid between open microchannels, and (e) to provide rapid shear-driven pumping. These are the necessary components for implementing chromatography at an unprecedented small scale.

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