2000

Atomic force microscopy of chemically modified fused silica capillary tubes

Paul Edward Pullen
San Jose State University

Follow this and additional works at: https://scholarworks.sjsu.edu/etd_theses

Recommended Citation

Pullen, Paul Edward, "Atomic force microscopy of chemically modified fused silica capillary tubes" (2000). *Master's Theses*. 2061.

DOI: <https://doi.org/10.31979/etd.65qm-9nsr>
https://scholarworks.sjsu.edu/etd_theses/2061

This Thesis is brought to you for free and open access by the Master's Theses and Graduate Research at SJSU ScholarWorks. It has been accepted for inclusion in Master's Theses by an authorized administrator of SJSU ScholarWorks. For more information, please contact scholarworks@sjsu.edu.

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

**Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600**

UMI[®]

NOTE TO USERS

Page(s) not included in the original manuscript are unavailable from the author or university. The manuscript was microfilmed as received.

95

This reproduction is the best copy available.

UMI

**ATOMIC FORCE MICROSCOPY OF CHEMICALLY MODIFIED FUSED SILICA
CAPILLARY TUBES**

**A Thesis
Presented To
The Faculty of the Department of Chemistry
San Jose State University**

**In Partial Fulfillment
of the Requirements for the Degree
Master of Science**

**by
Paul Edward Pullen
August 2000**

UMI Number: 1400674

**Copyright 2000 by
Pullen, Paul Edward**

All rights reserved.

UMI[®]

UMI Microform 1400674

**Copyright 2000 by Bell & Howell Information and Learning Company.
All rights reserved. This microform edition is protected against
unauthorized copying under Title 17, United States Code.**

**Bell & Howell Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346**

© 2000

Paul Edward Pullen

ALL RIGHTS RESERVED

APPROVED FOR THE DEPARTMENT OF CHEMISTRY

Dr. Joseph Pesek

Dr. Brad Stone

Dr. Paul Wagenknecht

APPROVED FOR THE UNIVERSITY

ABSTRACT

ATOMIC FORCE MICROSCOPY OF CHEMICALLY MODIFIED FUSED SILICA CAPILLARY TUBES

by Paul Edward Pullen

The 50 μm diameter bores of fused silica capillary tubes have been chemically modified to yield octadecyl derivatized surfaces with increased surface area. Alkylation of the native silica surface is achieved in three steps: etching, silane adlayering, and olefin hydrosilation. The surface roughness, surface area, surface-tip attractive forces, and topographic height images of the native, etched, silane-adlayered, and alkylated capillary bores are measured by atomic force microscopy.

Significant roughening occurs during the etching process, but the most dramatic change in roughness and surface adhesion forces occurs in the hydrided (silane-adlayered) capillary surface. A topographic smoothing and passivation of surface activity is observed for the alkylated surface. Scanning electron microscope images of surfaces from the same capillary samples compare favorably with the atomic force topographic height images.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Elizabeth and our children for encouraging me to pursue this research, and for enduring with patience during my coursework and seemingly endless hours in the lab and at the computer.

Appreciation goes to Prof. Paul Wagenknecht and Prof. Brad Stone for participating as members of this thesis committee, the time spent at seminar presentations, and for reviewing this document.

Thanks to Chuck Wade, who unknowingly motivated me to take time from a teaching career and pursue a master's degree; and to Vaughn Deline, who renewed my appreciation for good instrumental techniques. Acknowledgement with gratitude goes to the International Business Machines Corporation for providing the equipment and library resources for student research in current technology, along with the National Science Foundation, for supporting research programs that allow high school teachers and college students access to sophisticated experimentation.

My sincere thanks go to my thesis advisor Prof. Joseph Pesek, who focused me on the tasks at hand at just at the right times, and to Maria Matyska for instruction on capillary derivatization. My IBM research advisor Dr. Jane Frommer is especially thanked for providing much valuable time guiding me through the myriad of procedures inherent in atomic force microscopy, and illuminating interpretive elements that I would never have thought of. I would also like to thank my Brazilian colleague Paulo Herrmann, for instrumental instruction and special insight into the electronic basis for tuning an AFM cantilever.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	vi
LIST OF FIGURES	ix
LIST OF TABLES	xi
1. INTRODUCTION	1
1.1. Separation Techniques	2
1.1.1. Principles of Chromatography	2
1.1.2. High Pressure Liquid Chromatography	5
1.1.3. Capillary Electrophoresis	6
1.1.4. Capillary Electrochromatography	10
1.2. Chemical Modification of the Silica Surface	11
1.2.1. Previous Reaction Pathways	12
1.2.2. Silanization-Hydrosilation Reactions	13
1.2.3. Applications of Hydrosilated Surfaces	14
1.3. Methods of Characterization	16
1.3.1. Survey of Characterization Methods	16
1.3.2. Chromatographic and Electrokinetic Stability Studies	17
1.3.3. Chromatography Separation Data	18
1.3.4. X-Ray Photoelectron Spectroscopy and ESCA	19
1.3.5. CP-MAS NMR Spectroscopy	21
1.3.6. DRIFT Spectroscopy	22
1.3.7. Bulk Analytical Methods	23
1.3.8. Microscopic Techniques	24
1.4. Scanning Probe Microscopy	27
1.4.1. SPM Survey	27
1.4.2. Scanning Tunneling Microscopy	28
1.4.3. STM Applications	30
1.4.4. Atomic Force Microscopy	32
1.4.5. AFM Applications	33
1.5. Scanning Force Microscopy	34
1.5.1. Nature of AFM Tip-sample Forces	34
1.5.2. AFM Contact Mode Probe Mechanism	36
1.5.3. AFM Intermittent Contact Mode Probe Mechanism	39
1.5.4. AFM Non-Contact Mode Probe Mechanism	40
1.5.5. AFM Tip-Sample Force Measurement	41

TABLE OF CONTENTS (Continued)

1. INTRODUCTION (Continued)	
1.6. AFM Control and Detection System	44
1.6.1. AFM System Description	44
1.6.2. AFM Experimental Parameters	45
1.6.3. AFM Calibration	46
1.7. Review of AFM Research on Related Surfaces	47
1.7.1. AFM Topographic Imaging and Metrology	47
1.7.2. AFM Force Measurements	50
1.8. Statement of Research Objectives	52
2. EXPERIMENTAL	53
2.1. Apparatus	53
2.1.1. AFM Instrumentation	53
2.1.2. AFM Computer Hardware and Software	53
2.1.3. SEM Instrumentation	53
2.1.4. Capillary Modification Equipment	54
2.2. Materials	54
2.2.1. Chemicals and Supplies	54
2.3. Derivatization of Capillaries	54
2.3.1. Etching, Silanization, and Hydrosilation	54
2.4. Preparation of Capillaries for AFM and SEM Imaging	55
2.4.1. AFM Sample Preparation	55
2.4.2. SEM Sample Preparation	55
2.5. AFM Procedures	55
2.5.1. Intermittent Mode AFM Method	56
2.5.2. Contact Mode AFM Method	56
2.5.3. AFM Force-Distance Curve Acquisition	57
2.5.4. Surface Area and Surface Roughness Measurement	57
2.6. SEM Procedures	58
2.6.1. SEM General Procedure	58
2.6.2. SEM Parameter Settings	59

TABLE OF CONTENTS (Continued)

3. RESULTS AND DISCUSSION	60
3.1. AFM and SEM Visual Topographic Image Analysis	60
3.1.1. Native Surface Topography	63
3.1.2. Etched Surface Topography	63
3.1.3. Hydrided Surface Topography	66
3.1.4. Alkylated Surface Topography	68
3.2. AFM Surface Variation Measurements	69
3.2.1. RMS Roughness Measurements	69
3.2.2. Surface Area Measurements	70
3.3. Surface Force Measurements	71
3.3.1. Surface Force Distributions	71
3.3.2. Force-Distance Curve Profiles	73
3.3.3. Further Force Studies	75
3.4. Comparison of AFM Surface Roughness, Area, and Force Results	75
3.5. Correlation of Surface Morphology to Chromatographic Behavior	76
4. CONCLUSION	78
REFERENCES	79
APPENDIX A (Figures)	87
APPENDIX B (Table)	125

LIST OF FIGURES

Figure 1.	Optical micrographs of polyimide-sheathed fused silica tubing.	87
Figure 2.	Instrument configured for HPCE or OTCEC separations.	88
Figure 3.	Transverse section SEM image of a 20 μm i.d. etched capillary tube.	89
Figure 4.	Beam pathways in the AFM, SEM, and optical microscopes.	90
Figure 5.	Optical micrographs of a C_{18} -derivatized capillary tube surface.	91
Figure 6.	Electron cascade responsible for secondary electron production in SEM.	92
Figure 7.	Instrumental features of a field emission scanning electron microscope.	93
Figure 8.	Forces encountered as a SFM probe approaches a surface.	94
Figure 9.	Potential energy dependence on distance between approaching particles.	95
Figure 10.	Optical image of a silicon nitride AFM probe engaged with a surface.	96
Figure 11.	SEM micrograph of a silicon nitride AFM probe tip after use.	97
Figure 12.	SEM micrographs of an unused silicon AFM probe and tip.	98
Figure 13.	SEM micrograph of a damaged silicon AFM probe tip.	99
Figure 14.	Idealized AFM force curve and associated probe behavior.	100
Figure 15.	Instrumental features of an AFM.	101
Figure 16.	GC oven configuration for capillary modification.	102
Figure 17.	Transverse section SEM image of an etched CEC capillary tube.	103
Figure 18.	Force-distance curve measured on a native silica capillary surface.	104
Figure 19.	AFM section analysis of a native silica capillary surface.	105
Figure 20.	AFM height image comparison of alkylated curved and flat surfaces.	106
Figure 21.	SEM and AFM images of CEC tube wall surfaces at each of four stages in the derivatization process.	107
Figure 22.	AFM images of CEC tube wall surfaces at each of four stages in the derivatization process rendered in 3-D with z-dimensions as captured.	108

LIST OF FIGURES (Continued)

Figure 23.	AFM images of CEC tube wall surfaces at each of four stages in the derivatization process rendered in 3-D with enhanced z-dimensions.	109
Figure 24.	Topographic AFM images of native silica capillary tube surfaces.	110
Figure 25.	Topographic AFM images of etched silica capillary tube surfaces.	111
Figure 26.	Small scan AFM images of etched silica capillary tube surfaces.	112
Figure 27.	AFM phase image of etched silica capillary tube surface.	113
Figure 28.	Small scan AFM and SEM images of etched silica capillary surfaces.	114
Figure 29.	High resolution SEM image of etched features in a blocked tube.	115
Figure 30.	Low power SEM image of a hydrided capillary tube.	116
Figure 31.	Topographic and phase AFM images of hydrided capillary surfaces.	117
Figure 32.	Topographic AFM images of alkylated silica capillary tube surfaces.	118
Figure 33.	Small scan AFM and SEM images of alkylated capillary tube surfaces.	119
Figure 34.	RMS roughness histograms of the four stages of surface derivatization.	120
Figure 35.	Surface area histograms of the four stages of surface derivatization.	121
Figure 36.	Tip-sample force histograms of the four stages of surface derivatization.	122
Figure 37.	Topographic AFM image and correlated force curve for a specific (passivated) region on an etched silica OTCEC tube surface.	123
Figure 38.	Topographic AFM image and correlated force curve for a specific (activated) region on an etched silica OTCEC tube surface.	124

LIST OF TABLES

Table I. Summary of mean values and standard deviations of the RMS roughnesses (Rq), surface area % differences, and surface-tip adhesive forces for the four stages of capillary modification.

125

I. INTRODUCTION

Fused silica capillaries are routinely used in the separation sciences. These capillary tubes are often packed with porous, adsorbent materials to aid in the retention of targeted substances. Some modes of operation require that these packings be chemically altered for more specific separations. Professor J. Pesek et al of San Jose State University have developed improved chemical pathways for modifying solid porous materials. Furthermore, capillary tubes have been modified for separation methods, without the need for introducing packing materials. These modifications can increase the number of adsorption sites on the tube walls when the tubes are chemically etched, and more specific separations are possible by varying the final layer of material lining the tube walls.

New techniques and materials require theoretical, operational, and physicochemical description. The description presented in this paper is a physicochemical characterization of the modified capillary tubes using newer force microscopic methods. Although porous materials have been characterized extensively by Professor Pesek's group, the geometry and the fused state of capillary tubes have limited the characterization of the tube wall. Significant description of the surfaces of these tubes is left to inference from the porous packing material studies. Therefore, alternative analytical methods are needed to accommodate the tube configurations. Research herein uses a method of surface analysis that provides a description of surface areas, surface roughnesses, topographic features, and surface (chemical) forces of adhesion found on derivatized fused silica tube walls.

This manuscript describes: (1) the methods that utilize derivatized capillaries, (2) the earlier capillary derivatization reaction pathways as compared with those of Professor Pesek's group, (3) the existing analytical methods of characterization, (4) the principles of scanning probe microscopic techniques, (5) research reported in the literature that employ probe techniques on similar surfaces, and (6) the application of atomic force microscopy to characterize chemically-modified fused silica tube surfaces.

1.1. Separation Techniques

1.1.1. Principles of Chromatography. The methods of separation science are used to isolate materials. Once separated, molecules of interest may be analyzed or used to produce substances not easily attainable by other means. An example of a physical separation is the gas phase porous effusion of a mixture of $^{235}\text{UF}_6$ and $^{238}\text{UF}_6$ molecules, which allows the enrichment of rarer ^{235}U isotopes to be used in fission processes. Some separations depend mainly on chemical interactions coupled with thermal effects. For example, metals (e.g., Ni or Pt) are bonded to surfaces to form low-threshold energy, activated complexes with specific gases in catalytic converters.^[1]

The application of physical and chemical properties of matter for the purpose of separating substances is the basis for chromatography and its related methods. Tswett (1906) discovered that a plant leaf chlorophyll extract poured onto a column of adsorbent is resolved into colored bands, when followed by a solvent. Each band moves at a different speed, representing a component of the mixture.^[2]

In high school science, students use paper chromatography to learn that black ink actually consists of dyes with different colors. When a paper with an ink spot is suspended in a suitable solvent, individual colored dyes move up the paper with the solvent. It is often known that ink flows when it is wetted, but many students discover that the dyes move at different rates. Since the solvent (mobile phase) moves at a uniform rate, the dyes must be interacting with the paper (stationary phase). The distance a dye (analyte solute) travels divided by the distance the mobile phase travels in a given time is a measure of the dye's retention factor, k' . A more exact way of obtaining k' is to choose a distance (detection window) along the stationary phase and measure the amount of time required for the mobile phase and each analyte solute to reach the window. The time required for a given solute to reach the detection window (retention time) is a function of equilibration during a sorption-desorption process occurring between the solute and stationary phase. Retention time is used to qualitatively separate solutes in chromatography techniques, while methods used to determine how much solute is retained provide quantitative data.

The general definition used in chromatography for the retention factor (k') is given by

$$k' = (t_1 - t_0) / t_0 \quad (1)$$

where t_1 is the retention time for a given solute, and t_0 is the retention time for an unretained solute.^[3]

Chromatography literally means “graphing of colors”; however, the term refers to any separation based on retention factors.^[1] When substances are colorless, the position and intensity of the bands of solute are detected by spectroscopic methods. The position of a band of solute with respect to time, and its relative intensity in absorbance units of radiant energy is represented by a peak. A set of peaks representing the mixture is known as a chromatogram.^[3] The peak height, or more often the area under the peak, is used to find the quantity of analyte.^[4] The separation process may be based on adsorption, the distribution between phases,^[5] or the differences in rates of diffusion.^[6]

Molecules encountering a stationary phase are alternately sorbed and desorbed. Sorption limits the rate at which the molecules may move through the column, thus the elution position of a chromatographic peak depends mostly on its equilibrium sorption, k' . The width of a peak depends on kinetic and mass transfer processes (diffusion, flow, and turbulence). If the flow rate of the mobile phase is slowed through a column, sorption equilibrium is approached, and peak separation is enhanced. However, diffusion increases with lower flow rate, which causes the peak width to increase.^[7] Thus, the methods used to control kinetic effects and to maximize k' are vigorously researched in chromatography.

Each sorption and desorption equilibration is called a theoretical plate. The number of theoretical plates (N), is a measure of column efficiency for a given solute,^[3] given by

$$N = 16 (t / w)^2. \quad (2)$$

The term t is the solute peak retention time, and w is the peak width at a defined position on the peak. Equation 2 predicts that a narrow peak width indicates a more efficient solute separation. Finely divided stationary phases (i.e., with higher effective surface areas) allow increased equilibration rates and higher flow rates, thus producing better peak separation and finer resolution. When a stationary phase is packed more densely into a

column, more pressure is required for a suitable mobile phase flow rate. This task has been addressed^[7] by the technique of high pressure liquid chromatography (HPLC), using porous silica as a high surface area support material for more than three decades.^[8]

The fused form of silica has been used as capillary tubing in gas chromatography (GC),^[9, 10] capillary electrophoresis (CE),^[11] and capillary electrochromatography (CEC).^[12] A problem identified early on in electrophoresis is called zone spreading, in which the zones or bands of analyte are subject to thermal diffusion. High voltages required for good electroosmotic flow produce Joule heating,^[13] which cause the molecules in the warm center of a large diameter tube to move faster. Narrowing the diameter of capillary tubing^[13-16] helps to overcome Joule heating by narrowing the temperature differential between the center and wall of the tube. Additionally, heat is dissipated more rapidly.^[17] The dependence of the temperature differential (ΔT) on the tube diameter is given by

$$\Delta T = (0.239Q / 4k)r^2 \quad (3)$$

where Q is the power density in watts/m³, k is thermal conductivity, and r is the tube radius.^[14] Equation 3 predicts that while a 1 cm radius tube filled with water has $\Delta T = 4^\circ\text{C}$ when the potential is 10 V/cm and the current is 10 mA/cm², ΔT is reduced to 0.04 $^\circ\text{C}$ in a 0.1 cm radius tube under similar conditions. In a study of ΔT dependence on tube radius, it was found that a 50 μm i.d. tube had ΔT sixteen times lower than that for a 250 μm i.d. tube with like conditions.^[15]

Another improvement on capillary construction is the use of fused porous silica sheathed with polyimide, in place of glass. Glass is opaque to UV detection wavelengths < 280 nm, whereas fused silica is transparent to a wide range of UV and visible wavelengths.^[18] The addition of polyimide sheathing provides greater flexibility and strength than glass. Optical micrographs of silica capillary tubes (i.d. = 50 μm , o.d. = 375 μm) with intact polyimide coatings used in this research are shown in Figure 1. In comparison, an average human hair has a diameter of 100 μm .

1.1.2. High Pressure Liquid Chromatography. Normal liquid chromatography (LC) involves the separation of analyte solutes by a series of equilibrations between different phases present in a column. A variety of separation modes are used based on adsorption, partition, ion exchange, and size exclusion.^[4] Modes of application within the major techniques are more numerous. Normal phase, hydrophobic interaction, reversed phase, affinity and chiral selection are a few examples of methods used in the adsorption technique.^[3] The application of high pressure to a liquid chromatography column (HPLC) allows elution of a mobile phase through a densely packed stationary phase. Since each particle of stationary phase has a number of sorption sites, a greater number of particles and sites enhances the separation process, leading to well-resolved chromatographic peaks. Thus, stationary phase development is important for increasing the column efficiency predicted in equation 2. A quantity closely related to efficiency is the peak resolution (R), defined^[8] by

$$R = \Delta t / w_{av} \quad (4)$$

where Δt is the difference in retention times, and w_{av} is the average peak width of adjacent peaks. The surface area and particle size of the stationary phase is controlled through the use of porous silica, thus increasing the resolution.

Normal phase HPLC uses high pressure to drive a solvent through a column packed with a polar stationary phase, usually porous silica.^[19] The mobile phase solvent must be fairly non-polar to resist interaction with the support bed, and carries hydrophilic solutes through the stationary phase. A porous frit must be used prior to the analyte detector to prevent support materials from interfering with the analysis. Detection methods used for chromatographic and electrokinetic separations are numerous, chosen on the basis of specificity for the analyte, limits of detection, through-flow capability, convenience, and economic considerations; absorbance spectroscopy in the UV-vis range is most widely used.^[4]

Reversed phase HPLC is used more frequently than the normal phase technique.^[19] This mode makes use of chemically-modified (e.g., alkyl-bonded) porous silica as a hydrophobic support material, and the mobile phase utilizes a polar solvent. The reversed phase allows the separation of $\leq$ picogram quantities of non-polar substances from hydrophilic solvents, such as a mobile phase containing a phosphate buffer. This mode extends the LC technique to many classes of compounds and applications, including the determination of impurities in pharmaceuticals and pesticide levels in the biosystem.^[4, 20, 21]

Although porous silica has been used extensively in HPLC for the support material,^[22] other porous oxide surfaces are commonly used, e.g., alumina, zirconia, titania and thoria.^[19,23] Silica (and alkyl-bonded silica) is limited in hydrolytic stability^[24, 25] to the range $2 \leq \text{pH} \leq 8$, whereas alumina^[19] and other porous oxide surfaces^[23] are stable over a wider range of pH values. Modification schemes used to attach organic ligands to porous silica have provided a range of surface properties from hydrophobic to hydrophilic and ionic.^[22] Although alumina has been similarly modified for the purpose of reversed phase HPLC for some time,^[26] a surface containing a silicon-carbon bond resistant to hydrolysis has been applied.^[19] This surface provides enhanced stability and chromatographic resolution. The fused silica capillary tubes mentioned earlier are also amenable to the modification schemes used for porous silica surfaces.^[27]

1.1.3. Capillary Electrophoresis. When narrow bore ($\leq 75 \mu\text{m}$ i.d.) fused silica tubes are used in CE, the method is generally termed high performance capillary electrophoresis, or HPCE.^[22] Tiselius (1937) first described the method of free solution electrophoresis^[28] using large vertical u-shaped tubes. The technique was limited by zone spreading caused by Joule-heated convective diffusion. Small bore ($200 \mu\text{m}$ i.d.) teflon capillaries were introduced later,^[14] followed by the use of even smaller ($75 \mu\text{m}$ i.d.) glass capillaries by Jorgenson and Lukacs,^[16] which reduced Joule heating. Quenching the heat effect allowed higher voltages (30 kV) to be applied. Efficiencies of $N \approx 400,000$ theoretical plates were observed, compared to 20,000 theoretical plates being considered high for HPLC at that time.

Currently, the most widely used capillaries are 50 - 75 μm i.d. fused silica tubes, described earlier. Enhanced peak resolution is found when these tube surfaces are chemically modified with organic substances.^[29]

A CE experiment^[3] consists of introducing an analyte into a capillary tube inlet from a sample vial. The inlet is moved into a source vial, and the tube outlet ends in a destination vial, as shown in Figure 2. The capillary and the source and destination vials contain a buffered solution for the mobile phase. A high voltage potential is applied across an anode in the source vial and a cathode in the destination vial, causing separation of the analyte solutes by charge and size. A small section of polyimide sheathing is removed from the tube, to provide a window for an incident radiant energy detector beam. Sample introduction may be done via siphon action, pressure injection, or electrokinetically. Electrokinetic injection occurs by applying a field across the sample vial.

Pure silica has a fairly reactive surface, since it consists mainly of silanol groups.^[25] This reactivity can be beneficial since it is responsible for electroosmotic flow (EOF) in CE. The presence of a buffer solution with $\text{pH} > 3$ causes the formation of silanoate ions that interact with solution cations, forming a double (positive) electrical layer close to the surface of the capillary wall. The cation layer adjacent to the silanoate surface is fixed, and the inner layer is mobile. A shear plane exists within the double layer, and the potential difference along this plane is called the zeta potential. The zeta potential is proportional to the EOF.^[3] The inner mobile layer of cations is attracted to the cathode, causing a diffusion gradient that carries the buffer solution along the capillary. If the EOF cannot be controlled or minimized, the mobile phase flow rate may increase, resulting in Joule heating and deterioration of the buffer characteristics, discussed below. In the absence of EOF, the high voltage applied is responsible for electrophoretic mobility, hence, the sequential separation of solute cations, neutrals, and anions. The smallest, most positively charged cations elute first, due to greater electrophoretic mobility. Solute molecules travel through a buffer solution with an electrophoretic velocity, v_{ep} , which is a function of the electrophoretic mobility (μ_{ep}) and the applied potential. The EOF carries the

buffer solution with an electroosmotic flow velocity of v_{eof} , which is a function of the electroosmotic mobility (μ_{eof}) and the applied potential. Thus, the electrophoretic and electroosmotic flow mobilities are similar, and their velocities are approximately treated as either plus or minus direction vectors, analogous to a boat traveling up or down a flowing river. Without EOF, cations migrate to the cathode with velocity v_{ep} . With EOF, the cations migrate with the combined (observed) velocity of $v_{ep} + v_{eof}$. Neutral species migrate with v_{eof} , and anions migrate with an observed velocity of $v_{eof} - v_{ep}$ in the presence of EOF. Anions still migrate to the cathode, since EOF mobility is usually greater than electrophoretic mobility. It is possible to reverse the EOF by adding a suitable flow modifier such as a quaternary amine to the buffer solution, or by chemically modifying the capillary tube wall. In either case, the charged species in solution are prevented from interacting with the silanolate wall surface. Additionally, the polarity of the applied potential source may be reversed, such that the anode resides in the destination vial, allowing rapid separation of anionic solutes. The basis for separation in electrophoresis and the factors influencing mobilities may be summarized in the following equations.^[13]

The electrophoretic velocity (v_{ep}) of a charged solute moving through a buffer is given by

$$v_{ep} = \mu_{ep}E \quad (5)$$

where μ_{ep} is the electrophoretic mobility, and E the applied potential. The electrophoretic mobility (μ_{ep}) is given by

$$\mu_{ep} = q / 6\pi\eta r \quad (6)$$

where q is the charge of the ionized solute, η is the buffer viscosity, and r the solute radius.

Equations 5 and 6 show that the mobility is the capacity for charged species to move as limited by mass transport characteristics, whereas velocity includes the motive force.

Thus, solutes with greater charge and smaller size have greater mobility, and for a given buffer and applied voltage, a greater velocity.

The potential difference (ζ) between the double layer of cations is a measure of EOF, given by

$$\zeta = 4\pi\delta e / \epsilon \quad (7)$$

where δ is the thickness of the double layer, e is the charge per unit surface area, and ϵ is the dielectric constant of the buffer. The double layer thickness is inversely proportional to the buffer concentration. The velocity (v_{eof}) of the electroosmotic flow is given by

$$v_{eof} = \mu_{eof}E, \quad (8)$$

and the electroosmotic mobility (μ_{eof}) of the buffer is given by

$$\mu_{eof} = \epsilon\zeta / 4\pi\eta \quad (9)$$

where all quantities are defined in equations 6 and 7. Substitution of equation 7 into equation 9 shows that the electroosmotic mobility is dependent only on buffer characteristics, and is independent of the applied potential.

An advantage of EOF is that its flow front has a flat profile, insuring that solute particles will have uniform velocity components compared to the laminar profile of the pumped flow front in HPLC.^[3, 27, 28] EOF also responds favorably to applied potential as shown in equation 8, reducing analyte migration times proportionately.^[30]

The silica surface is sensitive to changes in pH. A plot of the effect of buffer pH on EOF at constant ionic strength has a similar profile to that for the dissociation of silica vs. pH,^[31] providing further incentive for chemical modification. Ionic strength at constant pH also has a significant effect on EOF. Generally, as the buffer concentration increases, the EOF decreases at constant temperature.^[32] If the temperature is not controlled, the EOF may increase due to an increase in current, and Joule heating can occur.^[33]

To summarize, the parameters of applied voltage, buffer pH, buffer concentration or ionic strength, temperature, presence of organic solvents, and the chemical nature of the capillary wall determine the factors (viscosity, dielectric constant, and zeta potential) that influence electrophoretic mobility and EOF. The EOF may be slowed, reversed, or even stopped altogether, by chemically modifying the capillary wall. This quality allows the selective separation of many classes of analytes.

1.1.4. Capillary Electrochromatography. Pretorius et al (1974) first described^[34] capillary electrochromatography (CEC). The technique was further developed by Jorgenson and Lukacs^[35] around the time they introduced the use of small bore glass capillaries to CE. In a CEC experiment, a column is packed with stationary phase material and a mobile phase is introduced, as in HPLC. However, the mobile phase and solutes are drawn through the tube by an applied voltage as in electrophoresis, eliminating the need for high pressure pumping. Thus, CEC is a hybrid of the HPLC and CE methods, and the resulting spectral absorption peak function of time is termed an electrokinetic chromatogram.^[36]

A variation of the CEC method uses micelles in the mobile phase to separate neutral molecules along with charged analytes.^[37] This method is analogous to reversed-phase HPLC, since partitioning of solutes occurs between a hydrophobic stationary phase and a hydrophilic mobile phase. Additionally, the elution order is similar; hydrophilic, polar solutes elute first, and hydrophobic non-polar species are retained the longest.

Problems identified with CEC have included the theoretical^[38] requirement of narrow bore ($< 100\ \mu\text{m}$) capillaries and submicron packing materials. However, it has been shown that Joule heating is reduced in capillary bores as great as $320\ \mu\text{m}$ due to high organic content in the mobile and stationary phases.^[39] Furthermore, electroosmotic flow is demonstrated to be independent of packing material particle size to $1.5\ \mu\text{m}$.^[40] Other problems include those inherent in the HPLC method such as bubble formation,^[39-42] packing difficulties^[43-45] and the use of frits.^[46, 47] The problems related to packing have been addressed by a theoretical treatment of the use of open (unpacked) tube capillaries

with modified walls.^[48] Open tubular capillary electrokinetic chromatography (OTCEC) has subsequently been demonstrated,^[49, 50] using the instrumental configuration employed by HPCE, as in Figure 2. The success of OTCEC in competing with conventional CEC depends heavily on the nature of the tube wall surface, since this configuration is attempting to substitute for the porous packing material normally used. The surface area of the packing materials in conventional CEC is generally high, $\geq 100 \text{ m}^2/\text{g}$.^[19, 20] A method of etching $50 \mu\text{m}$ i.d. fused silica tube walls similar to that described by Onuska et al^[51] has been used to produce walls with enhanced surface areas by Pesek and Matyska.^[27] Additionally, a method of bonding alkyl moieties to a porous silica surface via a direct Si-C surface bond was developed by Sandoval and Pesek.^[27, 52-57] This method was extended to fused silica capillary surfaces for use in HPCE and has been described for use as the stationary phase on capillary walls in OTCEC by Pesek and Matyska.^[27]

In summary, chromatographic techniques are improved by addressing modes of separation and studying the parameters that control those modes. These parameters are manipulated to produce an optimal separation of desired substances. Thus, the nature of the stationary phase surface is of primary importance in electrokinetic chromatography. The methods of stationary phase surface modification are examined below.

1.2. Chemical Modification of the Silica Surface

Several chemical reaction schemes can be used to bond organic moieties to the surface of porous silica.^[22] The characteristic shared by each of these pathways is the presence of silanol on the surface as the substrate reagent.^[8, 25] A non-mechanistic summary of four important methods used to bond organic groups to porous silica follows. The first three methods were in use before the silanization-hydrosilation scheme was developed to derivatize capillaries examined in this research. Bold-typed (Si) atoms in equations 10 -17 are understood to be surface-bonded.

1.2.1. Previous Reaction Pathways. The first reaction is a condensation between an alcohol and a surface silanol:

where R = a desired organic group. Generally termed esterification, this was the first method used for preparing alkylated support material for HPLC.^[58] The Si-O-C linkage produced on this surface is prone to hydrolysis^[22] in aqueous mobile phases.

A second scheme using organosilanization can occur by two pathways.^[59] The first method utilizes a single reactive group X on an organosilane, leading to a monomeric surface, and the second method utilizes three reactive X groups on an organosilane, leading to a polymeric phase at the surface. The first reaction is a condensation between the hydroxyl group on the surface silanol and the group X:

where X is a chloro-, methoxy- or ethoxy group, and -SiRR'R'' represents an organosilane with the desired organic groups R, R' and R''. The second reaction is essentially the same as in equation 11; however, the presence of three X groups produces bonding sites for the surface silanol and two potential cross-linking sites between adjacent organosilanes:

where X is defined as before, and Y = H, or Y = Si from either a second or third surface silanol, or an adjacent organosilane. The organosilane Si atom becomes the surface silicon if at least one Y group is an adjacent organosilane Si central atom. If three surface silanols were perfectly oriented, it is conceivable that a single organosilane may bind all three sites, making each Y moiety a silicon atom. A more likely situation is described by Y representing a central Si atom on an adjacent organosilane, forming what is referred to as a cross-linked polymeric phase. If R displays significant steric hindrance, then the second Y could become H, which leaves an incomplete coverage. The organosilanization scheme forms a Si-O-Si-C surface linkage,^[22] which offers significant improvement in hydrolytic stability over the esterification method. However, the Si-O-Si bonds in the first product (equation 11) are susceptible to aqueous phase attack, since no protective cross-linkage

exists. Resistance to hydrolysis by the product in equation 12 depends on the extent of formation of the polymeric phase.

The third scheme requires two steps, since the surface is chlorinated by thionyl chloride before a desired organic molecule is attached directly to the Si surface atom.^[60] Substitution of a chloride with organic may take place using either a Grignard reagent or an organolithium compound. Surface chlorination proceeds as:

and the chloro group is substituted with the desired organic R, using a Grignard pathway:

or in a reaction with an organolithium:

The resulting Si-C linkage forms a derivatized surface resistant to attack by water. The major drawback to this method is the requirement for an absolutely anhydrous preparation environment, since the Si-Cl bond is easily hydrolyzed.^[22]

1.2.2. Silanization-Hydrosilation Reactions. An alternative to the methods described above is a fourth scheme involving the formation of a hydrided silicon surface, followed by a hydrosilation step.^[22] Each surface silanol group may be reduced to silicon hydride with lithium aluminum hydride,^[52, 61] but a faster, more efficient approach is silanization of the surface with an alkoxy silane.^[54] Under carefully controlled conditions in the presence of an acid, usually HCl, a Si-O-Si(OY)₂ surface linkage is formed along with the triethoxysilane (TES) condensation product ethanol:

where Y = an adjacent silane under optimal conditions. Evidence supporting the complete coverage of the silanol surface with a silicon hydride adlayer is examined in the next section. For the moment, it is assumed that optimum coverage is present, and Si-O-Si cross-linking prevails. Thus, the new surface linkage Si-H becomes a direct Si-C linkage in the second (hydrosilation) step of this synthetic route.

The reaction proceeds in the presence of a suitable catalyst, such as hexachloroplatinic acid:

where R = the desired organic moiety, and Y is defined in equation 16. Since the silicon is more acidic than the hydride, Si is the electrophile in an anti-Markovnikov addition across the olefin double bond, thus forming a Si-C surface bond in a mechanism similar to a hydroboration reaction.^[62]

The structure of R has few limits, so the nature of the derivatized surface can be specifically tailored to the desired properties for a particular type of separation. Most often, the organic adduct is derived from a terminal olefin (e.g., 1-octadecene). Other precursors containing double or triple bonds such as alkynes, nitriles, or non-terminal olefins can be used.^[54]

1.2.3. Applications of Hydrosilated Surfaces. Examples of porous silica derivatization precursors employed in the silanization-hydrosilation scheme include the use of 1-octene (C₈) and 1-octadecene (C₁₈),^[52-57] 7-octene-1,2-diol (7-OD) and allyl glycidyl ether (AGE),^[63] a C₈ mono-ol, a butyl phenyl, and a perfluorinated C₈.^[64] A particularly interesting specialization of the surface involves bonding the liquid crystals cholesteryl 10-undecenoate and 4-methoxyphenyl-4-allyloxy benzoate (MPAB) to porous silica.^[22] These stationary phase liquid crystals exhibit enhanced selectivity based on molecular shape.^[65-69] The silanization-hydrosilation scheme (using R = 1-octene and R = 1-octadecene) has also been applied to the porous surfaces of alumina, zirconia, thoria, and titania.^[23] The major advantage of using these alternative porous oxides is their potential use as stationary phase material over a broader pH range than with silica.^[19, 22, 23]

Since capillary tubing currently in use is formed from fused silica as discussed earlier, the tubing surface is chemically similar to that of the silanol surface of porous silica. In theory, the silanization-hydrosilation method of derivatization also applies to these tubes. Although the tubes are used in HPLC,^[22] CE,^[70] and OTCEC,^[27] the open tubular (OTCEC) method is of particular interest in this research, since the technique draws from the qualities of both HPLC and CE. The use of an open tube allows the charge separation

and flow characteristics of CE, while the use of a modified stationary phase applies the specificity of derivatized porous silica used in HPLC. Unlike HPLC, both CEC and OTCEC require no pressure for the mobile phase,^[29] simplifying the equipment requirement. A major advantage is gained in OTCEC,^[71] since the problems of packed stationary materials^[39-47] are eliminated.

The high surface area of porous oxide stationary phase material is lost when using open tubes. However, increased sites on the tube wall may be formed under strict time and temperature controls by rigorous etching of the capillary bore with a hydrogen fluoride-like substance, ammonium hydrogen difluoride (NH_4HF_2).^[27, 51] Etching can produce radial extensions up to 5 μm long from the surface,^[29] which may become too thin to withstand mild mechanical stress if the etchant is too aggressive. Broken plates can lead to blockage, especially in very narrow bore tubes, shown in Figure 3.

The newly etched surface is intentionally activated under strong basic conditions, to provide a maximum of silanol surface sites. At this point, the silanization-hydrosilation reactions are carried out on the tube surface, as in equations 16 and 17. The actual derivatization differs only procedurally from the method used for porous silica. Details of the modification will be given in the Experimental section. For simplicity, the chemical steps required to carry out the silanization and hydrosilation of silanol groups on fused silica, porous silica, and other porous oxide surfaces will be termed *hydrosilation*.. Fused silica surfaces used in HPCE are understood to be derivatized directly, whereas those surfaces used in OTCEC are chemically etched prior to derivatization. When it is necessary to refer to the surface product of the silanization procedure, the term *hydride(d)* will be used; otherwise, a derivatized surface is understood to be the final organic product linked to the surface Si, regardless of the method or bond linkage.

1.3. Methods of Characterization

Some 50 or more analytical techniques are specifically designed to probe the surfaces of materials.^[72] The solid state spectroscopic techniques have limited utility in derivatized fused silica capillary tubes, due to limited accessibility and the geometry of the wall surfaces. However, both the porous and fused forms of stationary phase are amenable to chromatographic and stability studies. The characterization techniques used for porous surface analysis infer information about the nature of fused surfaces through chromatographic and electrokinetic methods of separation.^[22]

1.3.1. Survey of Characterization Methods. Characterization of chemically modified silica and other porous oxide surfaces is facilitated using information-rich spectroscopic techniques, due to the high surface area and particulate nature of the material.^[73] Some of the solid state methods used to probe hydrosilation-derivatized surfaces include diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy,^[19, 22, 23, 73] cross-polarization and magic angle spinning nuclear magnetic resonance (CP-MAS NMR) spectroscopy,^[19, 22, 23, 68, 73] ESCA or X-ray photoelectron spectroscopy (XPS),^[20, 73] optical microscopy,^[74] and scanning electron microscopy (SEM).^[27, 74] Some other techniques that are not surface-specific (i.e., bulk analysis), but provide information to help characterize these materials include thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), and plasma atomic emission spectroscopy.^[19, 23]

Surface roughening and increasing surface area of the OTCEC capillary bores is required for providing stationary phase material for solute separations, as discussed earlier. Quantifying these modifications is essential for optimizing the etching and derivatization methods. Since information regarding the nature of fused silica tubes and modified tube walls is often limited, it is evident that other methods of probing these surfaces are needed. The SEM technique of imaging the surface provides excellent visual information, but this method measures topography in only two dimensions. Also, non-conductive surfaces must be metal vapor-deposited, which alters chemical and physical properties.^[4, 75]

Atomic force microscopy (AFM) is a newer technique that allows 3-dimensional (3-D) surface measurements such as surface area and surface roughness, and *in situ* 3-D topographic imaging.^[72] The AFM method also provides surface chemical information from the precise measurement of forces of interaction between the probe tip and sample.^[4, 72, 76, 77, et al] The chromatographic techniques and solid state analytical methods are discussed below. The theory and methods of the scanning force microscopies are presented in the next section.

1.3.2. Chromatographic and Electrokinetic Stability Studies. One measure of successful porous silica derivatization is to determine how well the support material replicates the original separation data, after repeated elutions. Stable derivatized materials do not interact significantly with the mobile phase, and will retain the original surface bond linkage. This stability was tested^[55] by eluting polycyclic aromatic hydrocarbons (PAH) through HPLC columns packed with: (1) (C-18)-silica modified by the hydrosilation method, (2) (C-18)-silica modified with high organic coverage by the organosilanization method, and (3) (C-18)-silica modified by the organosilanization method, with low organic coverage. The fraction of the initial k' was measured for nearly 3000 injected volumes. Column 1 still had almost 90% of its initial k' , column 2 had just over 65%, and column 3 only retained about 5% of its original capacity. Similar results were obtained at high pH, which support the hypothesis that the hydrosilation method produces a hydrolytically stable Si-C linkage at the surface.

Derivatized fused silica tube surfaces have also been tested for stability using HPCE^[70] and CEC.^[27] In a HPCE experiment, polyacrylamide was compared with poly(acryloylaminoethoxyethanol) to determine retention efficiencies in protein separations. The poly(AAEE) was linked to the surface of an unetched tube wall using the hydrosilation method, whereas the polyacrylamide was bonded using organosilanization. After 300 elutions, the poly(AAEE) electropherogram was essentially unchanged, but the polyacrylamide protein peaks were either completely lost or unrecognizable.^[70] Further evidence for the stability of the hydrosilated Si-C linkage has been demonstrated with

OTCEC using an etched, C₁₈-derivatized capillary tube. More than 100 injections of several different samples in a variety of buffer conditions were followed by several methanol washings and dry storage. In an experiment using the same tube, electrochromatogram results of 31 consecutive injections of lysozyme followed by 31 consecutive injections of ribonuclease A showed no significant increase or decrease in retention times.^[22]

1.3.3. Chromatography Separation Data. Confirmation of surface coverage after derivatization, and an indication of the quality of that coverage (e.g., whether desired cross-linkage occurred) can be partly determined by the chromatographic behavior of the stationary phase. An example of this was shown by comparing the retention ($\log k'$) as a function of the hydrophilic nature of bonded stationary phases in fused silica HPLC capillary columns.^[20] A different substituted phenol was injected as the solute in each of nine experiments, with three series of experiments being carried out in the normal phase (heptane-chloroform mobile phase). The literature value for a commercially available diol (Merck)-, a lab-prepared (AGE)-, and a lab-prepared (7-OD)-derivatized column was used for each series respectively. The commercially prepared diol has the same structure as the AGE diol, but it is surface linked through an ether. The AGE and 7-OD diols were bonded via the hydrosilation method. The order of retention was (Merck) diol > AGE diol > 7-OD diol. These results can be interpreted to mean that the literature diol has residual silanol sites on the surface, in addition to a short alkyl chain (less hydrophobicity) and an ether surface linkage, thus exhibiting greater affinity for the phenolic (solute) groups. The AGE diol column has a reduced affinity for the solutes due to fewer silanol sites. This can be interpreted as better surface coverage due to hydride replacement. Further decreases in retention occur as the alkyl chain length is increased through the use of the 7-OD diol, increasing the hydrophobic effect observed with AGE. Summarizing, the hydrophobic trend (retention decrease) is enhanced by: (1) removal of the ether linkage, (2) decreasing the residual silanol groups on the surface, and (3) increasing the alkyl chain length.

Therefore, by determining the detrimental effects caused by hydrosilation and molecular structure in the context of the HPLC column k' , information regarding the attributes of the column surfaces was obtained.^[27, 29]

1.3.4. X-Ray Photoelectron Spectroscopy and ESCA. The acronym ESCA, i.e., electron spectroscopy for chemical analysis, is used to describe the photoelectron spectroscopy (PES) methods. These methods apply Einstein's theory of the photoelectric effect.^[78] Electron emission from an atom occurs if the threshold frequency of incoming radiant energy ($h\nu$) is sufficient to overcome the binding energy or work function (ϕ) of the atom,

$$h\nu = \phi. \quad (18)$$

Any photon energy beyond the threshold is translated into the kinetic energy of the emitted electron:

$$h\nu = \phi + 1/2mv^2 \quad (19)$$

where m and v are the mass and velocity of the photoelectron. The kinetic energy term in equation 19 is measured with the spectrometer, and $h\nu$ is known. In principle, the binding energy ϕ , unique for each element, is found by difference. In practice, the work function of the spectrometer must also be measured,^[72] and included as a term on the right of equation 19. If the incoming energy is UV, the technique is termed UVPES or simply UPS, and only valence orbital electrons are ejected. The method of Auger electron spectroscopy uses electrons for the primary beam. Valence ("Auger") and core orbital electrons, X-rays, and secondary valence electrons of the energy used for SEM imaging are emitted in a complex series of events known as an electron cascade.^[79] When X-rays are the primary beam, only core orbital electrons are ejected, and the method is known as XPS. The PES methods are called ESCA techniques, in honor of the method introduced by the 1981 Nobel Laureate in physics, K. Siegbahn. The XPS method is also called ESCA.

Since a specific electron orbital energy is unique for each element, ESCA is the method of choice for information-rich elemental analysis. The binding energy of an electron in its ground state (initial state prior to photoemission) is unique; however, this value is different if the ground state is influenced by chemical environment. The difference in binding energies is known as a chemical shift (not in the same sense as NMR), and may be used to help determine the structure of groups bound to the analyzed element.^[72, 78]

In a modification of porous silica using chlorination and LiAlH_4 reduction before hydrosilation, ESCA was used to evaluate the intermediate hydride surface.^[53] Unreacted silica had peaks for the 1s and 2s Si states on a partial ESCA spectrum. The reacted silica spectrum had four additional peaks, assigned to the 1s and 2s states of Cl and Al. After acid washing the support material, a third spectrum was produced, essentially identical to that of the unreacted silica.

A porous silica hydrosilation experiment^[20] used to derivatize the surface with AGE produced a gray-colored stationary phase when an excess of the catalyst hexachloroplatinic acid was used. The HPLC chromatogram of a mixture of steroids eluted through the AGE support material indicated peak broadening and significant peak tailing (i.e., asymmetry due to strong adsorption). Another chromatogram under like conditions using clean (white) AGE-derivatized material produced sharp peaks with good separation, indicating that residual Pt catalyst in the gray AGE silica was interacting with the solutes. A Pt 4f region ESCA scan was taken of the native silica and hydride intermediate, revealing no detectable Pt. Pure Pt catalyst was scanned in the same region, producing j-j (spin-orbit coupled) split subpeaks:^[72] $4f_{5/2}$ near 77 eV, and $4f_{7/2}$ near 73 eV. The gray AGE material was similarly scanned, with noisy but recognizable subpeaks: $4f_{5/2}$ near 75 eV, and $4f_{7/2}$ near 71 eV. This evidence means that excess platinum catalyst as Pt(IV) was probably reduced to elemental Pt by hydride groups present at the beginning of the hydrosilation step.^[20] This is consistent with PES chemical shift theory^[72, 78] which predicts a more positive binding energy for higher oxidation states. Scans made in the chlorine 2p region confirmed that Cl in the gray AGE material was not coordinated with the Pt(IV).

1.3.5. CP-MAS NMR Spectroscopy. The surfaces of interest in the silanization-hydrosilation method include mostly silicon- and carbon-centered materials, and to a lesser extent, aluminum.^[73] The isotope ^{12}C is the most abundant form of carbon, but an even number of protons and neutrons gives ^{12}C zero nuclear spin, spin moment, and magnetic moment.^[80] Since a magnetic moment is required for resonance techniques, only isotopes such as ^{13}C , ^{29}Si , and ^{27}Al can be detected in the NMR analysis of the surfaces studied here. Natural abundances of the above isotopes are 1.11%, 4.70%, and 100%, respectively;^[17] thus, the signal will be very low for the ^{13}C and ^{29}Si NMR techniques. One development designed to enhance the NMR signal is cross-polarization (CP). This has the effect of transferring magnetization from abundant nuclei, such as protons, to low abundance isotopes. Another advancement in solid state NMR is magic angle spinning (MAS), in which spinning of the solid material at a 55.3° angle imitates the motion of an anisotropic liquid.^[81]

The CP-MAS NMR method was used to provide chemical information pertaining to porous alumina surfaces derivatized by the hydrosilation method.^[19] The ability of NMR to resolve specific chemical groups was demonstrated by measuring the chemical shifts of the ^{29}Si , ^{13}C , and ^{27}Al nuclear spins. An example^[19] is the ^{29}Si CP-MAS NMR spectrum of (a) silanized alumina and (b) hydrided, C_{18} -derivatized alumina. Spectrum (a) included a major peak at -85 ppm assigned to Si-H, and two minor peaks: one at -110 ppm assigned to a Si atom with four siloxane bonds, and one at -100 ppm assigned to a Si atom with three siloxane bonds and one silanol bond. Spectrum (b) included a new peak at -65 ppm, attributed to the Si-C octadecyl linkage, and a decrease in the Si-H peak at -85 ppm. The -100 ppm peak was still prominent and the -110 ppm peak was also present, indicating either impurities in the starting reagent or degradation of the hydrided surface before hydrosilation took place. Further evidence is necessary in this case, which may be provided by the technique of DRIFT spectroscopy, discussed below. An example of a different type of NMR experiment is given in the analysis of surfaces derivatized with liquid crystals as described earlier. These substances exhibit enhanced selectivity based on

molecular shape,^[65-67] attributed to bonded phase transitions as measured by an increase in NMR spin-spin relaxation times (T_2^*) when the temperature is lowered.^[68] The bonded phase liquid crystals change their degree of association, similar to molecular reorientations which occur in pure liquid crystalline materials.^[22] Conversely, a decrease in T_2^* is observed with lower temperature in the case of C_{18} , indicating restricted molecular motion.^[69]

1.3.6. DRIFT Spectroscopy. The signal measured in NMR is due to nuclear spin response to an increasing magnetic field scan over a steady or pulsed (radio) frequency. The electromagnetic radiation spectroscopies (e.g., IR) collect absorption or transmission signals using an increasing frequency scan.^[62] Frequencies detected in the time domain are Fourier transformed to the frequency domain^[62, 72, 80, et al] in units of wavenumber, cm^{-1} , made possible in DRIFT by the use of Michelson interferometry.^[73]

In the diffuse reflectance (DRIFT) technique, highly particulate samples scatter light. The probe beam is angled such that a diffuse reflected beam is directed back to an ellipsoidal surface, then on to a detector. This method depends on the theory that only diffuse scattered light has penetrated the surface and sampled the material.^[72] A distinct advantage of DRIFT over other methods is the ease of sample preparation and short analysis time.^[19, 73] Often, no sample preparation is required; for example, direct surface analysis of pharmaceutical tablets is routine.^[4]

Alumina surfaces discussed in the NMR section above were also analyzed by DRIFT spectroscopy.^[19] Native alumina was scanned first, showing no remarkable absorbances except for some small bands attributable to several Al-OH groups. This region is complex, because alumina exists as a mixture of roughly 20% belonging to the T_d symmetry point group, and the rest as O_h .^[82, 83] Secondly, a hydrided alumina scan showed a large band at the Si-H stretching frequency of 2250 cm^{-1} . In a third scan, the C_{18} -derivatized alumina surface had a diminished, but still prominent Si-H band in addition to strong C-H stretching frequencies between 2800 cm^{-1} and 3000 cm^{-1} , which are also

found on derivatized silica.^[22] Also present were Al-OH bands with nearly the same intensity as those on the native alumina, inferring incomplete coverage. Information regarding the unreacted -OH groups on these surfaces was obtained using the analytical techniques discussed in the next section.

1.3.7. Bulk Analytical Methods. These methods are destructive, i.e., materials either have part of their structure changed, or the structure is lost altogether. Plasma emission spectroscopy is based on detecting the spectrum of emitted energies from materials in highly energetic states.^[4] An inductively coupled argon plasma is a suitable energy source, from which the emission passes onto a photodetector in the spectrometer. Quantitative analysis of silane adlayered onto the alumina surfaces discussed above was found by emission spectroscopy. The silicon layer(s) were stripped from the surface using a strong base.^[19] A combination of the emission spectrum results, calculation of the Brunauer-Emmett-Teller (BET) surface area,^[80] and the mean particle size provided by the alumina manufacturer allowed the calculation of the Si surface coverage. Thus, the number of silanol sites available for silanization was determined.

Thermogravimetry uses a balance that senses changes in weight of a material as a function of temperature.^[4] Analysis of the alumina by TGA revealed that only 38% of the silanol sites calculated above were actually reacted. This result was interpreted^[19] to mean that the reagent TES has restricted access to the OH group due to steric considerations.^[84]

Differential Scanning Calorimetry (DSC) measures the enthalpy change associated with a chemical or physical change.^[4] The difference between the energy introduced into a sample and that of a reference (usually air) is measured as a function of temperature as the sample and reference are either heated or cooled. The hydrided alumina from the above modification was analyzed for thermooxidation using DSC.^[19] A single prominent energy minimum occurred near 525 °C. The alumina hydride surface appears to be chemically bonded as opposed to physically sorbed compared with hydrided silica. Silica oxidizes near 440 °C, and thermooxidation of the polymerization product of neat TES (polyhydrosiloxane) is near 365 °C.^[54]

1.3.8. Microscopic Techniques. Optical microscopes have been used for extending the power of human vision for about 400 years, around the time that Galileo built the first astronomical telescope.^[85] The resolution of sample features is as important as the power of magnification.^[79] In 1775, Henry Baker^[86] cautioned microscopists to “Beware of Determining and Declaring your Opinion suddenly...Pass no Judgment upon Things over-extended by Force, or contracted by Dryness, or in any Manner out of their natural State without making suitable Allowances.”

Light microscopy uses visible light to form images of objects with the aid of glass lenses, as in Figure 4. The magnification range is limited to 1000 times the numerical aperture, i.e., $\approx \times 1300$. Theoretical resolution is limited to $0.5\lambda \approx 2000 \text{ \AA}$, where λ is the wavelength of blue light.^[4] Although transmission bright field microscopy is the most commonly used method, some techniques make use of a dark field of vision, reflected light, polarization, phase contrast and interference, and other variations.^[87] Examples of phase contrasted and reversed polarization angle optical micrographs of a C_{18} -derivatized capillary are shown in Figure 5.

An optical microscope is a convenient tool for rapidly scanning prominent surface features $> 0.5 \mu\text{m}$.^[4] Such features have been compared in native and etched fused silica capillary tubes.^[74] Radial extensions inside an etched tube that became detached when the tube was mechanically stressed were visibly resolved. Thus, gross feature progress of the etching step in capillary modification may be confirmed quickly with inexpensive, readily available equipment. A low power dissection microscope used for AFM and SEM sample preparation was indispensable in the current work.

Scanning electron microscopy^[4, 75, 79] uses accelerated electrons (1 - 30 keV) as a probe, analogous to incident light (see Figure 4) in optical microscopy. The source beam and condensor lens coils in SEM are inverted with respect to the sample, compared to an optical microscope. The electron beam is focused and scanned over a conductive, opaque sample surface. If the sample does not conduct electrically, it must be coated with a thin film of suitable conductor (e.g., Au). In rough analogy, a reflected beam is produced.

Actually, the primary beam of electrons interacts with the surface according to energy states. This interaction produces secondary electrons, backscattered primary electrons, a few Auger electrons, and very few low energy X-rays in a cascade fashion,^[72, 75, 80] shown in Figure 6.

A diagram of the essential features of a SEM are shown in Figure 7. The secondary electrons form the majority of the (reflected) image beam, and are attracted to an anodic scintillator. The electron energies are converted to photons, which are photomultiplied into electrical signals proportional to electron intensity. The photomultiplier also modulates cathode ray tube (CRT) intensity. A scan generator controls the magnetic scanning coils and CRT simultaneously, so that an image of the scanned sample is produced on the CRT with virtually no lag time.

The electron microscope requires a sophisticated vacuum system, contributing to its relatively high initial and operating costs. All internal chambers must be under vacuum during scanning, and pressures range from 7×10^{-4} Pa (sample chamber) to 1×10^{-7} Pa in the electron gun chamber. Sample introduction is facilitated via an airlock system.

Sample preparation for SEM consists of mounting the specimen on a disk using double-faced graphite adhesive tape, to provide a dark, conductive substrate. If the sample is non-conductive, e.g., silica, the specimen and disk are placed into a chemical vapor deposition (CVD, or sputtering) chamber. An argon ion beam is used to bombard a metallic target (e.g., Au, Pt, Pd) of high purity under high vacuum, and a plasma state is produced. Vapor phase metal ions are attracted to the specimen disk, made to be cathodic with respect to the target, and the elemental metal is deposited over the entire disk.^[88]

The practical magnification range of a typical SEM is $\times 15$ - $\times 400,000$, and the limit of resolution is ≈ 20 Å.^[4] The depth of focus in SEM is superior to most other methods, including optical microscopy, transmission electron microscopy (TEM) and AFM.^[89] With sputtering capability, almost any type of solid sample may be imaged by SEM within the size constraints of the sample mount. Some organic materials may polymerize under the electron beam, but this effect is localized and does not affect the overall topography.^[79]

When strongly insulative materials are scanned, a surface charging occurs that shows as white on the image, which frequently blocks important features. This can be reduced by resputtering the sample, or using an instrumental parameter known as gamma adjustment as a filter. Backscattered electron signals are unaffected by charging; thus, a variation of SEM incorporates this property. Since backscattered electrons are detected in the line of sight, an image may be enhanced by minimizing the tilt of the sample stage.^[75] Many other parameters (e.g., accelerating voltage, condensor lens current, objective lens aperture size, et al), control the quality of the image, similar to those in optical microscopy. The production of secondary electrons (thus, the signal) is maximized when the accelerating voltage is minimized; however, resolution is maximized at higher voltages. Thus, certain factors that influence signal intensity and quality must be compromised to suppress noise, as encountered in most instrumental methods.

Allowing the cost and perceived complexity, SEM is one of the most reliable and rapid methods for extremely well-resolved visual surface information, and is used routinely in most solid state materials analyses. Starting with an unmounted sample, it is reasonable to produce a range of magnified images in less than one hour. In this research, it was found that the probing capability of the electron beam contributed to simplified sample preparation. Small transverse sections of capillary tubing could be sliced off with a tempered razor blade, sputtered, then imaged. These sections are not accessible with a normal AFM probe. Limitations found in SEM include those listed above, and the inability to produce simultaneous 3-D measurements, as discussed earlier. Also, surface preparation (i.e., sputtering) destroys the chemical nature of the sample. Therefore, SEM is mostly used to provide additional information to primary methods of analysis. Some chemical information is obtainable using the method of energy dispersive spectrometry (EDS or EDX, since the signal measured is produced by X-rays). This technique is limited to measuring X-ray intensities of elements $Z \geq 8$, produced when the electron beam has a high (10 - 30 keV) accelerating voltage.^[75] Analysis requires additional detector equipment, and extensive calibration for only semi-quantitative work.^[79] The techniques of

ESCA, plasma emission spectroscopy, and Auger electron spectroscopy (AES) are considered to be superior for elemental analysis.^[4, 72, 79]

A study using SEM^[27] was carried out on the surfaces of fused silica OTCEC capillary tubes in the native, pre-modified stage and those etched as described earlier. Visual analysis of tube walls etched under varying conditions allowed optimization of the etchant concentration, and the etching time and temperature requirements for the modification. The SEM technique was used in the current research to confirm the visual topographies of OTCEC tube surfaces in each stage of development, i.e., native, etched, hydrided, and C₁₈-alkylated, that were imaged by AFM using similar scan sizes. Additionally, the x,y-plane measurements of individual features were confirmed. A comparative discussion of the image qualities is included in the Results and Discussion chapter.

1.4. Scanning Probe Microscopy

Scanning probe microscopy is the family of techniques used to provide the topography features and physical (and chemical) properties of surfaces resolved down to the atomic level.^[72, 90, 91] The SPM techniques stem from two primary methods, STM and AFM. Techniques whose probe relies on electron tunneling current fall into the STM category, and those relying on surface force interactions are part of the AFM method.

1.4.1. SPM Survey. The AFM probe usually consists of a tip mounted perpendicularly on a substrate-mounted cantilever. However, an electrochemically sharpened metal wire is mounted directly to a control mechanism in the case of STM.^[89, 92] Imaging is facilitated through the interaction of the tip and sample in all of the SPMs,^[93, et al] and the mechanism of this interaction is the basis for the large variety of techniques in SPM. The technique of STM has been applied to surface imaging (e.g., adatom defects in Si[111]), surface manipulation (displacement of single atoms) and surface modification (lithography and chemical vapor deposition, et al). STM has also been used for atomic level instrumental techniques, with applications in spectroscopy and electrochemistry.^[90]

Atomic force microscopy senses surface forces; hence, the possible variety of techniques in this method of SPM is proportional to a like number of forces that can perform work on matter. Attractive and repulsive forces (e.g., van der Waal's, electrostatic, capillary, and friction) are at work on the AFM tip when normal imaging and surface force measurements take place.^[76] The method of imaging is complicated by whether the mode of scanning takes place with the tip in contact, intermittent contact, or without contact with the surface.^[94] Further variations depend on the tip chemistry, the springiness of the cantilever, and the medium in which the experiment takes place.^[91]

A more thorough survey of basic SPM principles may be found in a treatment by Frommer.^[91] For a historical perspective on the SPM drive and detection systems, an earlier review by Sarid and Elings^[94] introduces the mathematical basis for SPM control, and many of the specialized techniques developed during the prolific (6000 publications) twenty-six month period preceding 1996 are reviewed by Bottomley et al.^[90]

1.4.2. Scanning Tunneling Microscopy. The first SPM technique^[95] (STM) was invented in 1982, which merited the Nobel prize in Physics shared by Binnig and Rohrer in 1986. The STM may be visualized as a stylus profilometer^[4] incorporating quantum tunneling theory,^[72] controlled by advanced piezoelectric technology.^[91]

In use, the STM tip is lowered to within a few Ångstroms of the sample surface, a potential is applied, and current flows through insulative space to a conductive sample connected as ground. The insulator can be any non-conductor, including air or even a vacuum.^[72] Thus, the electrons are said to tunnel between the conductive surfaces. If a non-conductive material image is desired, (e.g., certain polymers) a thin layer can be adsorbed onto a conductive surface. Even though electrons are tunneling *through* the material, the surface is imaged. This observation affords a glimpse into the curious model of the tunnel junction being a hybrid state of the two electrodes (sample and tip) and the sandwiched non-conductor.^[91] There are two major modes of operation: constant voltage with current being measured, and constant current with instantaneous tunneling current measured. In the first mode, the tip is scanned over the sample surface as the tunneling

current is measured and the potential (bias voltage) is held constant between the tip and sample. Each current measurement is a data point used to construct an image, pixel by pixel. Since tip movement is controlled by a piezoelectric crystal, the exact tip position (to 0.1 Å) in the x,y-plane is monitored by the voltages applied to the piezo. The second and most common method measures the instantaneous tunneling current at each new position, and a bias voltage is applied to correct the current to a predetermined value, known as the set point. The piezo is calibrated in the z-direction, so that the displacement is known (to 0.01 Å) for a given change in bias voltage. In this instance, the z-displacement is plotted over the x,y-position, and an image is constructed from all of the data points.^[72, 89]

The physics of quantum tunneling applied to STM is not trivial, since the tip is not a one-dimensional point. However, a three-dimensional treatment of the wavefunctions facilitating tunneling current has been given by Tersoff and Hamann.^[96] The Schrödinger solution to an electron incident on a one-dimensional infinitely thick potential barrier (insulator) gives an imaginary term and a real term. The imaginary term is neglected, since it rises to infinity. The real term allows electron penetration where it is forbidden in classical mechanics. Thus, if the potential barrier is sufficiently narrow, there is a real probability of finding the incident electron inside, and this probability increases exponentially as distance inside the barrier decreases.^[97] The atomic level of sensitivity in the measured current is provided by this exponential dependence of the tunneling electrons on the distance between two conductors (tip and sample).^[91]

Since the electric potential on the surface of a spherical electrode is inversely proportional to the radius of the sphere,^[80] the discussion above becomes a handwaving argument if the STM tip does not approach atomic sharpness. Indeed, the ability of the tip to resolve is limited by the tip size and geometry. Consider a blind-folded person given the task of describing the size, shape, and surface texture of a group of rocks with a variety of sizes and shapes set on a table. A further restriction is that the person is only allowed to use the tip (without depressing the surface) of one index finger, which must be kept vertical at all times. As the probe of the unknown rocks begins, the description is frustrated by

limited tactile sensing. Any points lower than the vertical height of the outermost (horizontal) boundary of each rock are impossible to probe, regardless of the size of the rock. Perhaps more importantly, rocks that are smaller than the tip of the finger can only be recognized to exist. Quantitative data is limited to the height above the table, and topographic visual information for the small rocks is missing altogether.

In the context of STM, information is being shared between the sample and probe, i.e., both surfaces experience the tunneling current. Therefore a simultaneous sampling or *convolution* of current densities in the tunnel is monitored during the instantaneous tunneling current measurement.^[98] If the tip radius is less than or equal to the radius of the object (i.e., atom or molecule), the object will be imaged. If the object is smaller than the tip, the portion of the tip involved in tunneling will be imaged. In a practical sense, a probe tip is not likely to have only one atom at the apex. A more likely scenario is the confinement of a narrow stream of electrons between a small group of atoms on the tip and a small group of atoms on the sample.^[91] The ability to image and manipulate single “gas” atoms of Xe or CO, and even “solid” Pt atoms on a clean metallic surface with STM^[99, 100] seems to contradict this tip model at first. The question of what is actually seen is elusive. A local density of states (LDOS) approach contributes a partially satisfying explanation.

1.4.3. STM Applications. Tsukada et al^[101] used the linear combination of atomic orbitals (LCAO) method to calculate the electronic structure for a graphite surface, and for the STM tip based on a cluster of 10 tungsten atoms with a W[111] geometry. The atom count in the (outer to inner) first to third respective layers of this tip configuration number 1, 3, and 6. The structure of graphite is assigned two adjacent non-equivalent carbon atoms, A and B, in the top layer. Carbon atom A has another carbon atom lying directly below it in the first sublayer, but the first atom directly below carbon atom B is in the second sublayer. Corrugations observed in a STM image reflect the LDOS at the Fermi level in this model, instead of the positions of the atoms. The LDOS is lower at site A than it is at site B in this case, and the tunneling current maximizes over carbon atom B. Thus, a hexagonal lattice is observed instead of a honeycomb. If the apex atom of the tungsten tip

is removed, a triangle of atoms remain on the W[111] face, and the STM image is altered, which maximizes tunneling current over atom A. The second case is interpreted to mean that tunneling current is maximized when the combined current of the three equivalent W atoms is greatest, which occurs when the center of the tip is positioned over site A. Each of the three W atoms are centered over a carbon atom B in this case. If the center of the tip with three W atoms is centered over site B, each W atom is positioned over the center of a graphite hexagon, and tunneling current is minimized. In either scenario, a single atom tip apex or a triangle apex of three atoms, an image is produced with single atoms resolved. An excellent STM image of the hexagonal pattern on graphite may be seen in reference 91. The position of every second carbon atom is clearly seen, and explained by Fermi level LDOS being higher at β -sites (carbon atoms without atoms directly below) than at α -sites. Interestingly, when a full (stoichiometric) monolayer of graphite was deposited on platinum, there were still only 50% of the graphitic carbon atoms revealed in the STM image.

Other SPM techniques used in the STM method include scanning tunneling spectroscopy (STS) of LDOS. Current-voltage (dI/dV) measurements of tunneling current in phase with modulated voltage of π -orbital energies can be atomically resolved on Si[111] surfaces.^[102] Also, current imaging tunneling spectroscopy (CITS) of *local* tunneling current-voltage curves, e.g., images of Si[111] adatom bond-, dangling bond-, and backbonded states, have been resolved.^[103] Electrochemistry (EC) has benefitted from the ability of the STM to measure current and voltage, leading to the technique of ECSTM.

The STM has also been used as an electrochemical instrument in a technique known as scanning electrochemical microscopy (SECM). In this mode, voltammetry and other methods used in conventional electrochemistry are carried out at the microscopic level.^[90] The latent qualities of STM are inferred in the simultaneous use of the machine as an instrumental technique (i.e., SECM), and an imaging microscope (i.e., ECSTM).^[104] An example of this was demonstrated by using the STM for producing and imaging cathodoluminescence from single quantum dots.^[105]

In summary, it is evident that STM imaging is far from being a simple visual reflection of features positioned on a surface. Instead, a convolution of the (tunneling) stream of tip and sample surface electronic information is gathered and left for the investigator to interpret. Like the reflected beam in SEM, the image may be deceptively simple, belying the true source of information. Even so, STM is at the center of a revolution in fundamental research, in spite of uncertain theoretical foundations. The stunning images of the IBM logo formed by sliding xenon atoms with a STM tip over a nickel surface,^[99] and a “quantum corral”^[106] formed by standing electron waves exemplify the STM capability to probe matter at the level of its chemical building block.

Bulk analytical techniques such as NMR and UV/vis spectroscopy provide reliable molecular structural information using Fourier transformed data averaged over large sample populations, with individual features possibly overlooked. STM analysis of a few molecules of benzene reveal the molecule’s bonding and molecular dimensions, bonding angles, its local density of states, and perhaps its bonding or adsorption mechanism with the substrate.^[90, 91, 72] Before abandoning the NMR spectrometer, the STM proponent must know *a priori* that the molecule is benzene.

1.4.4. Atomic Force Microscopy. Binnig, Quate, and Gerber^[77] used a diamond fragment as a tip mounted on a cantilever to serve as a SPM probe, in the same year that the Nobel prize was awarded for STM. A STM mounted above the cantilever measured microscopic deflections of the cantilever in the z-direction, in response to a non-conductive sample scanned horizontally with a piezo control beneath the tip, using no tunneling current. Only natural forces between the tip and sample surfaces were at work as the probe was scanned in contact with the surface. Thus, the new technique (AFM) yielded images related to surface electronic energies up to the Fermi level. In contrast, the STM yields images related to surface electronic energies at or near the Fermi level.^[94] It was found that both of the SPM methods could be used to probe samples in a vacuum, air, or denser media such as water or organic solvents. Optical deflection measurements have replaced the STM for detection of the cantilever deflection in AFM.^[71, 89, 91, 94]

1.4.5. AFM Applications. Similar to STM, the AFM has been adapted to techniques other than simply imaging the cantilever's response to topographic surface-tip force convolutions.^[90, 91, 107] For example, by sampling the surface at a low frequency, the tip can interact normal to the surface long enough to plot either the cantilever deflection or the force required for the piezo to maintain constant deflection, as a function of probe z-displacement.^[76, 89, 108] In this measurement, a force-distance curve in the form of a hysteresis loop is produced. The cantilever deflection is due to the interface of surface and intermolecular forces found between the sample and tip,^[91] corresponding to values in the range of 10^{-9} to 10^{-6} N,^[72] although sub-picoNewton measurements are reported.^[109] Attractive and repulsive surface forces may be classified under the phenomena of adhesion, friction, and capillary action. Intermolecular forces may include the van der Waal's group of dispersion forces, and hydrogen bonding descriptions between particles in the condensed states.^[1] Capillary forces are due to the presence of a fluid layer of air containing (humidity) water and contaminants during ambient studies.^[76, 91, et al] A diagram of some of the surface forces acting on an AFM tip and the distance between the tip and surface where the forces are at work is shown in Figure 8.

Other techniques in AFM include longitudinal and lateral force measurements of friction, which give additional surface energy information.^[108] AFM measurements normal to the surface can also detect magnetic surface forces (e.g., on a storage disk) with a mildly magnetized tip, and charge-induced surface electrical potentials can be mapped.^[94] These variations of AFM are known as friction force microscopy (FFM), magnetic force microscopy (MFM) and electrostatic force microscopy (EFM), respectively.^[91] When a special restriction is placed on an AFM technique, it may also achieve status as an individual technique (e.g., lateral force microscopy (LFM) and (CFM), or chemical force microscopy).^[90, 110] Thus, efforts to unambiguously describe the techniques based on force measurements has led to the generic term scanning force microscopy (SFM).^[90, 91, 107]

The STM and SFM modes have been used in the analysis and manipulation of materials relevant to a spectrum of disciplines. These applications range from the observation of interactions between single DNA strands^[111] found by attaching oligomers to the tip and sample, to the molecular origins of friction and lubrication on computer hard disks,^[108] found by measuring the strong initial tip-to-sample attraction (i.e., capillary force) of liquid polymers.

1.5. Scanning Force Microscopy

This section deals with theoretical and applied principles of force microscopy leading to the measurement of topographic features and surface adhesion forces, and imaging surfaces using contact and intermittent contact AFM. The resolution in AFM is generally lower than that found in STM,^[72, 91] although single atoms confirmed by semiconductor defects have been resolved.^[112] In AFM, this resolution is achieved through tip-sample spacings corresponding to the repulsive region of the Lennard-Jones potential dependence ($1/r^{12}$) on distance.^[1, 80, 91]

1.5.1. Nature of AFM Tip-Sample Forces. The net forces experienced by the tip and sample can be divided into those in the attractive region, i.e., as the tip approaches the surface, and those in the repulsive region, after contact is made. A thorough treatment of the forces experienced between particles of various descriptions is beyond the scope of this study. However, a brief description of one of the microscopic force behaviors between a probe tip and sample helps to clarify the basis for SFM measurements. A generalized potential energy diagram^[72, 80, 89, et al] showing the relative magnitude and distance of forces as two spherical particles approach (e.g., atoms) may be seen in Figure 9. In the region below the zero of potential, the *net* forces are attractive. At r_0 , the potential is minimized, corresponding to the spatial position with maximum stability (neither attraction nor repulsion exists) between the particles. As the interparticle distance decreases, repulsive forces increase. The net forces of repulsion become positive just after intersecting the zero potential point.

Sarid and Elings^[94] presented a unified theory incorporating electrostatic, magnetostatic, and van der Waal's interactions to describe the drive systems used in SFM. The molecule-molecule van der Waal's work function, $W_{(z)}$, of the surface of a spherical tip with radius R separated from a plane parallel sample surface by distance z , is given by

$$W_{(z)} = - C / z^6 \quad (20)$$

where C is the dispersion force interaction energy, which incorporates both attractive and repulsive terms.^[80] Given the Hamaker constant A ,

$$A = \pi^2 C \gamma_1 \gamma_2 \quad (21)$$

where γ_1 and γ_2 are the interfacial energies of the tip and sample, the atomic force energy of interaction between an atom on the tip of the probe and an atom on the surface becomes

$$W_{(z)} = - AR / 6z. \quad (22)$$

A plot of this work function gives an idealistic potential energy curve for the non-contact mode of SFM. When a tip and sample approach the contact mode region in SFM, the Lennard-Jones molecular interaction energy model defines the work function as

$$W_{(z)} = 4\epsilon[(\sigma / z)^6 - (\sigma / z)^{12}] \quad (23)$$

where ϵ is the depth of the potential well, i.e., the energy at r_0 in Figure 9; σ is the distance between tip and sample when the potential $V = 0$ in the region of closest approach, and z is defined as before. By integrating equation 23 over the separation of the tip and sample, and equating the force of a deflected cantilever to that of the atomic force, then a complete force-distance curve may be constructed by plotting the potential energy dependence on the tip-sample distance,^[94] as predicted by

$$k(z - u) = 2/3\pi^2\epsilon\gamma_1\gamma_2\sigma^4R [(\sigma / r)^2 - (\sigma / r)^8]. \quad (24)$$

In this equation, z is the deflected cantilever position, and u is the undeflected (free air) cantilever position. The z terms on the right side of the equation have been replaced by r , to emphasize that this represents the separation distance of the atoms on the tip from those

on the sample, as derived from Lennard-Jones theory. The term $(z - u)$ is the vertical displacement of the cantilever in response to surface forces, k is the spring force constant of the cantilever, and the right side of the equation represents the atomic force. Therefore, equation 24 can be equated to a restricted (limited force treatment) form of Hooke's Law:

$$k\Delta z = F \quad (25)$$

where the ratio of the stress (force, F) to the strain (displacement, Δz) is a constant (k).^[17] The stress in this case is the surface-tip convolution of forces acting on the cantilever, and the strain is the displacement of the cantilever from its equilibrium (free air) position. In actual use, the tip is subjected to a range of forces as it approaches, and it experiences the sum of those forces as the tip engages the surface. Hence, equation 24 is an idealized model for a narrowly defined group of forces.

As in STM, the AFM is limited in resolution by the sharpness of the probe tip, which depends on geometry (aspect ratio) and radius of the tip. Macroscopic vs. microscopic tip properties are difficult to define, due in part to the tremendous pressures (GPa range)^[91] being applied locally, and the difficulties that arise from measuring the probe dimensions.^[107] Even when these uncertainties are accounted for, the AFM technique provides reasonably interpretable data on small (to atomic level) sample populations.

The AFM has the capability to produce a visual image of tracked non-conductive surface features of microscopic proportions. This capability also allows the AFM to measure its probe position in 3-D space, leading to surface roughness and surface area data with limits of detection in the realm of STM. These capabilities and the surface energy information gained through localized force measurements are the basis for the current research.

1.5.2. AFM Contact Mode Probe Mechanism. Force microscopy depends on the deflection of a cantilever in response to tip and sample interactions. This tip must be sufficiently small as to interact with the surface features of interest, and the cantilever must be flexible enough to respond to the forces of interaction. The degree of flexibility within

the elastic limits of a material is measured by Hooke's Law, in equation 25. Since a cantilever approximates a leaf spring, k becomes a cantilever spring constant in reference to the SPM methods. Thus, the choice of a probe material (e.g., silicon vs. silicon nitride) is based on the mechanical and chemical properties of the probe, the hardness and roughness properties of the sample, and the mode of the AFM experiment.

In the contact mode, the probe is more or less dragged over the surface, with height information from the surface sent through the degree of deflection of its cantilever. The cantilever designed for surface contact has a very low spring constant, so that tip-sample forces are less than sample molecular bonding forces. Surmising, the topography of the sample is measured without the displacement of surface atoms or deformation of softer materials.^[77] However, atoms can be intentionally displaced by the AFM.^[112] Surface topography can be influenced by strong compressive forces in response to the applied force normal to the sample, and by shear contact forces as the tip is scanned parallel to the sample.^[113]

Two methods of collecting scanned data are used in the contact AFM mode. The first is known as the *constant force* or slow scan method, where data is obtained by keeping the deflection of the cantilever constant via applied z-piezo voltage. The z-piezo voltage is used to produce the image. The second version of contact AFM keeps the z-piezo at a constant position relative to the sample, and the cantilever deflection is measured to produce a topographic image. This mode is known as the fast scan method, since the z-piezo is slow to respond to topographic features when either a faster scan or a slower feedback loop is utilized.^[114] On the instrument used in this study, the slow scan image is called height data, and most closely resembles a normal topographic image. The fast scan image is called deflection data, which resembles a phase image as described below, under intermittent contact techniques. The instrument used here produced images in the height and deflection data modes simultaneously.

If the contact AFM mode is needed for high resolution imaging, or if surface force measurements are being acquired, a soft cantilever is chosen, i.e., one with a low spring constant.^[89] Commercially produced cantilevers of this type are usually made with silicon nitride (Si_3N_4), are $\approx 100 - 200 \mu\text{m}$ long from substrate to tip, and have a nominal spring constant range of $k \approx 0.01 - 1.0 \text{ N/m}$. An optical micrograph of a silicon nitride cantilever engaged with the surface of a derivatized capillary bore is shown in Figure 10. A $4 \mu\text{m} \times 4 \mu\text{m}$ Si_3N_4 tip used in contact with hard (quartz and mica) surfaces is shown in a SEM image in Figure 11 to illustrate the pyramidal shape of the tip, and the nature of tip wear after normal but heavy use. The probe is constructed using microlithography techniques, and vapor deposited with gold for detector light reflection.^[91]

In a contact mode AFM experiment, the sample and the probe are mounted, and the detector is aligned such that an optimized signal is produced in coordination with the probe and sample relative motion through an electronic feedback loop. The instrument used in this study keeps the probe substrate fixed, and the sample is moved in the x-, y-, and z-directions by a 3-D piezoelectric control unit. Thus, only vertical deflection or torsional cantilever motion occurs with the probe in the contact mode. The probe holder is clamped inside an optical head, which contains a laser diode positioned over the probe cantilever, and the probe is manually positioned over the sample region of interest. A sample is suitably adhered to a round puck, which is held to the scanner magnetically. Although a video system is mounted over the optical head on the instrument used here, coarse positioning is also aided by a low power light microscope mounted on a pivoting clamp system. After coarse sample positioning, the detector signal is optimized and the starting parameters for the experiment are entered at a (computer) control console. Finally, the tip is engaged with the surface, and the control parameters are fine-tuned to optimize the signal, while minimizing the contact force.^[89, 91, 108]

1.5.3. AFM Intermittent Contact Mode Probe Mechanism. When an intermittent contact with the surface is desired, as is the case for visual topographic imaging and general morphology measurements over a wide range of materials and scan sizes ($<1 - 125\ \mu\text{m}$), a stiffer (higher k value) cantilever material is chosen. Silicon is usually the material employed for these cantilevers, which are $\approx 100 - 200\ \mu\text{m}$ long $\times 30\ \mu\text{m}$ wide. Scanning electron micrographs of the lower face of a silicon probe mounted on a substrate, and the pyramid shape of an unused tip on the same probe are shown in Figure 12. The spring constants of these cantilevers range from < 1 to $100\ \text{N/m}$. Contact and intermittent contact probes use the same type of holder, which is held stationary with respect to the piezo scanner in the instrument used in this research.

Intermittent contact AFM is a technique that utilizes high frequency cantilever oscillation at or near the cantilever resonance. The probe holder contains a piezoelectric bimorph that is independent from the sample position control unit. This piezo is used to drive the oscillation of the cantilever at its resonant frequency ($\approx 250 - 450\ \text{kHz}$) in the intermittent mode. The intermittent mode can be described as a vibrating probe in the non-contact, attractive region of a force vs. tip-sample distance curve, which was described earlier in a potential plot. The probe also regularly samples the surface in the repulsive, contact region, similar in method to obtaining force curves in the contact mode, but at a higher rate of acquisition. An advantage gained by using the intermittent mode is the ability of the tip to rapidly pierce and escape from the fluid air layer. This ability effectively minimizes slowed response of the cantilever due to capillary forces. Also, strong contact forces are minimized, which may otherwise introduce surface defects in the sample and tip.^[89] Such a tip defect produced by excessive contact force on a hydrided capillary tube is shown in a SEM photo in Figure 13. Note the point remaining in the middle of the damaged region. This tip artifact was still imaging some features fairly reliably; however, uncontrolled oscillations most likely occurred when the tip encountered tall, steep features, producing multiple images of blurry columnar-shaped objects. Preparing the sample and probe for intermittent contact AFM is similar to the contact mode, except a cantilever

oscillation tuning procedure follows optimization of the detector signal. The probe is then engaged with the surface, and an image is produced. The oscillating cantilever is detected optically in the same manner as with the contact probe, except the *amplitude change* caused by cantilever deflection is monitored in this mode. Two methods of data acquisition can be used in intermittent mode AFM, analogous to the height (slow scan) and deflection (fast scan) contact modes. In the height method, the data corresponds to the displacement voltage required for the z-piezo to maintain a constant oscillation amplitude, which is compared over the tip-to-sample distance during each measurement cycle with a predetermined set point voltage. The height method image is most frequently used for topographic imaging. In the deflection method, the z-piezo height with respect to the surface is held constant, and the change in amplitude (phase) of the cantilever is measured directly. The deflection, or phase signal is not used for topographic imaging as often as the height signal, since the cantilever deflection does not respond to tall features rapidly. This mode can provide valuable information on flatter surfaces, often confirming authentic vs. artifact features. Also, surface steps are better resolved with this method,^[89] and the presence of materials of different composition or crystal plane orientation are further revealed using a phase image. Similar to contact AFM, the height and deflection data is recorded simultaneously, and concurrent images may be viewed during an image capture.

1.5.4. AFM Non-Contact Mode Probe Mechanism. A non-contact mode AFM circumvents the problem of probe damage to soft samples. Martin et al^[109] developed an AFM method that keeps the probe tip in the attractive region of the potential curve, as a result of probing only longer range forces. Consequently, the minimized applied force is often overcome by the fluid air capillary force, causing two problems that are difficult to control. First, the feedback system must act in a reverse manner, i.e., the cantilever and sample are moved closer when the attractive force is reduced, and strong tip contact often occurs. Secondly, the thickness of the contamination layer can vary with surface topography, humidity changes, and sample warming.^[114] Advances in AFM control systems may renew interest in non-contact imaging and long-range force measurement.

The effects of capillary fluid layer forces have been addressed in the contact and intermittent contact methods with the use of a controlled fluid environment (e.g., water or buffered solution), known as a fluid cell.^[89] The fluid cell has opened many possibilities for samples of all types when the strong capillary forces of a contamination layer are reason for concern. Applications have included physiological *in situ* probing of biologically active substances.^[90, 107, 111, 113]

1.5.5. AFM Tip-Sample Force Measurement. Quantitative information regarding the chemical nature of a surface is inferred by localized adhesive and friction force measurements.^[110 et al] Tip-sample force interactions are determined by measuring the amount of cantilever deflection as a function of cantilever-sample separation^[89] in a cycle of tip-sample approach, contact, and retraction.

A drawing of a force-distance curve is shown in Figure 14 to illustrate the effect of sample adhesion and elasticity on the AFM probe. Approach and retraction cycles have been separated for clarity. Cantilever deflection has nominal units of distance or detector voltage (i.e., force), and the z-piezo position has nominal distance units which represent the absolute distance between the scanner position and the fixed end of the cantilever. Since the scanner is moved vertically to the probe, the up direction is negative on the abscissa. The probe and sample begin at maximum z distance, with zero deflection (i.e., free air equilibrium) at position 1. As the scanner and sample approach the probe, the tip may experience long range forces and deflect into the attractive region of the force curve (negative direction) at position 2. If no long range forces exist (e.g., the contamination layer is absent), the approach line continues horizontally, until the scanner and probe are moved as a unit into the repulsive (positive) cantilever deflection region. In the presence of a substantial fluid layer, the tip is strongly attracted to the surface with very little change in scanner position, as shown by position 3. This phenomenon is known as “tip snap” or “jump in”.^[89, 115] Following tip snap, the scanner and adhered tip move further (up) in the negative z-piezo direction. The cantilever passes through the zero deflection point (where attractive and repulsive forces are balanced) into the repulsive region. When the furthest

negative z position (and voltage) is reached, the cantilever has maximum positive deflection, as shown at position 4. If the material is soft, the probe tip may indent the sample. At this point the retraction cycle begins, and the cantilever steadily returns to the zero deflection point shown at position 5, while the tip remains adhered to the sample surface. The cantilever deflection at this position is equivalent to free air equilibrium (zero deflection), due to forces of repulsion and attraction being equal again. The separation distance between the fixed end of the cantilever and the sample surface is still fairly close at point 5. The difference between this distance on a hard surface and that on a soft surface is an additional indicator of the soft material's elasticity, discussed later. The retraction process continues past the zero deflection point into the attractive region, with the tip still adhered to the surface through attractive surface and intermolecular forces. Position 7 represents the point where the spring restorative forces in the cantilever overcome the forces of adhesion between the tip and sample, and the cantilever returns to the zero point of deflection (free air equilibrium) position almost instantaneously. No further cantilever deflection occurs, and the retraction is completed before a new force measurement takes place. If substantial long range intermolecular forces exist, the line from maximum negative deflection (point 7) to the free air position will not have infinite slope, and may even curve somewhat if capillary forces from ambient conditions are present.

Force curve analysis reveals many facts about the tip and sample system. The presence of a large tip snap infers strong long-range capillary forces at work on the tip. If the tip is hydrophilic (e.g., with silanol sites), these forces are probably due to surface tension between the tip and sample caused by natural humidity and contaminants in the fluid air layer. Mate^[108] used the presence of the tip snap minimum to measure the presence and thickness of bonded vs. adsorbed perfluoropolyether, used in storage disk lubrication. Tip snap was present on the force curve of a liquid layer of lubricant on Si[100], similar to the force curve of clean Si[100]. Conversely, bonded perfluoropolyether showed only a gradual increase in attractive forces. These results infer that the molecular description of lubricant failure is partly explained by unbound water

molecules reducing the shear strength and microcontact areas of lubricant molecules. Correlated friction measurements corroborated this interpretation.

The slope of the force curve retraction line from point 4 to 7 will change if the tip presses into the surface molecules, as compared with hard wall contact.^[89, 92] The slope predicts that a softer material will have less deflection, due to compliance with the applied contact force.^[116] This compliance can be measured using a lateral force mode. Young's modulus of elasticity^[17] has been determined for glassy polymers by Yang,^[117] and elasticities have been determined for biomolecules such as lysosyme^[118] and potentially wide-range application materials such as fullerenes.^[119]

Adhesion forces give the most pertinent chemical information available from the SFM techniques.^[90, 107, 110, 116] Force studies that differentiate between different materials based on interfacial energies from tip-sample adhesion data are numerous. These studies have been approached using native and derivatized combinations of hydrophilic and hydrophobic tips and samples. Experimental environments have included dry air or vacuum, normal ambient air, water and other hydrophilic solvents, and various organic solvents with several ranges of hydrophobicity.^[107]

Chemical information attained by these methods enables molecular level verification of models based on bulk analysis or other systematic studies.^[116] However, conclusive interpretations using force studies exclusively may not be altogether reliable. Fujihira et al^[120] cited examples of different monolayers of Langmuir-Blodgett films of like materials having different friction forces. Differences in forces on the same sample surface were observed on anisotropic monolayer surfaces with different sliding directions. Clearly, even localized phase differences will affect surface properties; hence, the adhesive, friction, and elastic forces on chemically identical surfaces can vary.^[121]

The adhesive surface force measured by AFM (see Figure 14) is determined from a force curve. The *vertical* distance along the cantilever deflection (ordinate) between positions 5 and 7 represents the cantilever deflection^[116, 120] due to tip-sample adhesive forces. This deflection data must first be converted to force data, which is complicated by

the simultaneous cantilever and scanner motion in response to attractive tip-sample forces and the z-piezo voltage, respectively. The data can be simplified by converting the z-piezo position to a tip-sample separation distance. This is accomplished by measuring the z-piezo positions at zero deflection when the tip and sample are adhered (position 5) and at maximum deflection when the tip is breaking free from the surface (position 7).^[89, 92, 116] This distance is defined as Δx , the total cantilever displacement under the influence of surface adhesive forces.^[122, 123, 124, 125] Thus, the adhesive force F_{adh} is found by

$$F_{adh} = k\Delta x. \quad (26)$$

The cantilever spring constant must be determined exactly whenever absolute force measurements are carried out.^[90, et al] AFM probes that are produced commercially on a wafer have a nominal force constant given, usually in addition to a range of resonance frequencies and cantilever length and width dimensions.^[126] Calculation of the spring force constant has included measuring the unloaded resonance frequency by mechanical or thermal drive, and by measuring the dimensions of a probe with known mechanical and chemical properties by SEM imaging. The uncertainties^[107] introduced by these methods frequently overlap the range of given nominal force constants. The use of frequency distributions (i.e., histograms)^[122, 124] and limiting comparative data to the same probe^[107] was the strategy used herein.

1.6. AFM Control and Detection System

Precise mechanical control of positioning equipment is accomplished through the use of piezoelectric materials, as discussed earlier. Piezo crystals used to drive SPM scanners or probes can respond to applied voltages in increments as small as 0.01 Å.^[90] The AFM detection and feedback systems are equally important in order to derive full advantage from the piezo control and sensitivity of the probe.

1.6.1 AFM System Description. A drawing of the essential features of the AFM system used in this research is shown in Figure 15. A 5 mW laser source in the optical head produces a beam directed down onto the probe cantilever. The cantilever

reflects the beam to an adjustable mirror, which allows optical alignment of the beam with a quad photodiode array. The quad array is known as a position sensitive detector (PSD). An amplified differential signal between the upper and lower quadrants of the PSD measures cantilever deflection, and a like signal between the left and right quadrants measures lateral changes in the cantilever. Assessment of these signals is accessible on the upper and lower liquid crystal displays (LCDs) found on the AFM motor housing. The LCDs display data from multimeters that are linked to the control station, PSD, x- y-, and z-piezoes, step motor, and the piezo bimorph. The type of signal displayed depends on whether contact or intermittent contact AFM is in operation.

Optical alignment is achieved by first positioning the scanner-mounted sample under the probe (level with the horizon) using coarse adjustment screws, followed by finer adjustment with the step motor. Signal gain is registered on a SUM elliptical gauge on the lower LCD. This SUM signal (literally, the sum of the PSD output) is maximized by adjusting the laser and mirror positions. If the intermittent mode is in use, the cantilever oscillation amplitude signal is maximized by the same procedure.

1.6.2. AFM Experimental Parameters. All other adjustable parameters, including the command for the tip to engage the surface, are carried out from the control station.^[127] In contact mode AFM, the x,y- and z-scan size and rate parameters are entered, and the set point and PSD output voltages are confirmed to be near or below zero (minimized contact force), and around -2.0 V, respectively.^[89] The set point voltage is pivotal in the control scheme of the AFM. In the contact mode, feedback electronics rapidly compare the desired set point voltage with the PSD output voltage, then apply an appropriate potential to the z-piezo to complete a required position change. Thus, the cantilever deflection and contact force is controlled via the set point value and set point amplifier gain controls. These gain controls affect the response of the z-piezo to the compared PSD voltage. If the gains are high, the z-piezo responds to keep the PSD output close to the set point, which is the desired effect in a constant force experiment where height imaging is sought. Low gain values have the opposite effect, and the primary signal

is due to deflection data, as in the fast scan method. An analogous description may be made for intermittent mode AFM, except that the set point is compared with the cantilever driver RMS oscillation amplitude voltage.

When intermittent contact AFM is to be used, an additional cantilever tuning step is required before engaging the probe with the sample. A scope mode (built-in oscilloscope) is included on most commercial AFM instruments,^[114, 127] which is used for fine tuning the z-scan start and z-scan size voltages, calibrating the z-piezo response, and tuning the cantilever frequency response, as a few examples. The cantilever must oscillate at or just below its resonant frequency with a controllable amplitude to produce a strong signal without overtone oscillations or excessive noise.^[127] The nominal resonance frequency of the probe given by a manufacturer is used as a starting drive frequency. The scope is used to find the frequency sweep curve on a plot of cantilever response (amplitude) vs. center frequency. The drive frequency is usually found to produce the best cantilever amplitude performance around 75-85% of the center frequency amplitude, i.e. before the peak of the frequency sweep.^[89] Once the tip is engaged with the sample, the above parameters may be adjusted in real time using the control station.

1.6.3. AFM Calibration. Data collected in any of the SPM methods depends on reliable knowledge of the position of the tip with respect to the sample. Thus, the applied x,y- and z-piezo voltages must be calibrated to correspond with displacement prior to using a new scanner. Additionally, a step motor used for positioning the scanner in the z direction requires calibration. The response of the scanner position to the piezo voltage may be periodically confirmed with appropriate calibration standards.^[89] The use of a calibration standard also contributes confidence in the quality of an AFM tip.

Well-defined surfaces, e.g., mica and diffraction gratings, and microlithographed surfaces, are used to calibrate the AFM. The frequency of calibration depends on the desired data acquisition. Absolute quantitative data requires parametric calibration for every new sample and every new probe tip,^[89, 93] whereas general topography imaging and comparative data trends require only occasional calibration.

1.7. Review of AFM Research on Related Surfaces

Reference to the AFM study of native or derivatized capillary tube walls was absent from the literature prior to circa 1994 - 1996. Indeed, most characterizational studies were operational (i.e., chromatographic or electrokinetic).^[128-132] However, considerable research was carried out on flat silicon surfaces in the fields of semiconductors,^[90] polymer coatings,^[91, 133] and biomolecular research.^[90, 107] Zhong et al^[134] imaged the outer surface of a fused silica optical fiber to probe the source of a polymer coating failure. Also, a novel approach to measuring surface forces on porous silica in nonionic surfactant solution was reported by Rutland and Senden.^[115] Wenzler et al^[123] deposited porous silica onto glass surfaces and AFM tips before derivatizing these surfaces by organosilanization. More recently, a few studies have been reported using AFM to directly probe the bore walls of fused silica capillary tubes.^[135, 136]

1.7.1. AFM Topographic Imaging and Metrology. Imaging flat, slightly rough surfaces with the AFM is forthright. The corrosion of silicon to silicon dioxide (silica) in the presence of air^[137] provides such a surface. Very smooth flat surfaces (i.e., mica) are simple to engage with the AFM tip, but imaging atomic-height corrugations requires special precautions against mechanical and sound vibrations, and noise sources such as stray electrical and magnetic fields.^[89] Very rough surfaces are also sensitive to external noise. When rough surfaces with sharp edges contain significant bow and warp,^[138] engagement of the tip with the surface becomes challenging. Imaging rough surfaces similar to the etched and hydrided surfaces in this research requires careful tuning of the machine parameters.

Disadvantages of the AFM method in the imaging mode include difficulties in physically accessing regions of convoluted surfaces and uncertainties in rendering non-planar surfaces.^[139] The latter problem arises from contributions of the nonlinear action of the scanning piezo to the translation of surface curvature.^[89] However, previous AFM studies^[135, 136] have shown that some of the problems associated with non-planar and non-uniform surfaces of coated (polyacrylamide) capillaries can be overcome.

Images found in the literature which were useful for comparative purposes in this study included naturally oxidized,^[140] chemically oxidized^[137, 141] and H-terminated Si[111] surfaces,^[137] octadecyl-derivatized silica (oxidized Si[100] wafers) and glass by organosilanization,^[140-143] time-lapsed corrosion of freshly cleaved glass,^[113] etched quartz,^[143] native fused silica optical fibers,^[134] and native^[135, 136] and polyacrylamide coated fused silica tube walls.^[136]

Topographic measurements are accessible by the AFM in three dimensions, which provide a distinct advantage over SEM. Other advantages mentioned earlier are the ability to detect surface forces, and an absence of destructive surface (conductive coating) preparation. The ability of AFM to provide accurate height information enables the determination of surface roughness and surface area data.^[89] Open tubular capillary electrochromatography (OTCEC) relies on increased surface areas and general roughening of the tube walls, which provide substrates for derivatization of stationary phase material, as discussed earlier.^[27, 29, 74] The etching processes used on fused silica capillary walls mentioned above^[135, 136] and naturally oxidized^[140] Si[111] are mild. The bare silica layer as oxidized on Si[111] is only about 20 Å thick, with a root mean square roughness of 5 Å.^[140] In a study in which microlithographed quartz surfaces contained alternating areas of chromium film and bare quartz, Fujihira and Morita^[142] were able to derivatize the exposed quartz with a C₁₈-organosilane. After this step, the chromium layer was etched from the quartz, and the remaining lithographed patterns were derivatized with a perfluoro-organosilane. The etched surfaces revealed oblongated pitting observed by AFM topographic imaging and friction force imaging. Ando et al^[137] imaged the etching process (0.68 M NH₄F) of Si[111] *in situ* using AFM. Elongated structures were present on the Si[111] oxidized surfaces, similar to those observed using SEM imaging^[27] of CEC tubes etched with NH₄HF₂. The origin of these structures was studied by imaging bare (freshly etched) unoxidized Si[111], which revealed the same structures. This suggests that the bare (etched) silicon topography provides a foundation structure for the oxide layer in this case.^[137] Further etching of the oxidized surfaces produced 0.3 nm high step-edged

structures normal to the [112] direction with triangular holes of equal depth. As etching proceeded, the lateral hole dimensions increased, and step edges receded. Orientation suggested that etching occurred laterally in the [112] direction. In a similar study, Ando et al^[144] observed previously H-terminated Si[111] surfaces. These surfaces appeared to be rougher in terms of large and small feature frequency. The features provide important visual information for comparative analysis in the research here on hydrided surfaces.

The algorithmic routines available for rendering AFM topographic height data into root mean square (rms) roughness values are incomplete at best. The rms roughness, R_q is calculated from a mean surface level,

$$R_q = \left[\sum_{i=1}^N (z_i - z_m)^2 / (N - 1) \right]^{1/2} \quad (27)$$

where N is the number of data points (pixels) in the image, z_i is the height of the i th pixel, and z_m is the mean image height.^[138,145] The roughness parameter is difficult to interpret, since R_q is dependent on the scan size, 3-D resolution, and sampling density.^[145, 146] Some uncertainty may be removed by statistical (distribution) plots, but the height variations over lateral distance within samples must also be considered. The absence of lateral information oversimplifies the rms calculation to represent averaged height variations without consideration of spatial frequency variations. An algorithm that addresses this absence of lateral information is the power spectral density (PSD) curve. However, this method can still “overlook” certain high spatial frequencies, as found in non-uniform surfaces with substantial bow and warp.^[138]

Simpson et al^[145] evaluated the quality of rms roughness measurements taken by contact vs. intermittent contact mode AFM. Gaussian distributions of rms roughness heights were observed for both modes. Correlation of the R_q standard deviation (σ) with R_q showed that the intermittent mode of AFM is clearly more precise ($r^2 = 0.994$) than the contact mode ($r^2 = 0.376$).

The surface area increase observed on etched fused silica capillaries by SEM imaging^[27] can only be guessed in best circumstances. Height imaging by AFM allows the calculation of an increase in surface area via a surface area ratio^[89] of the image 3-D surface area to the threshold plane 2-D image area. The 3-D surface area is determined by summing the triangular areas of all triangles formed by any three adjacent pixels on the image. As with R_q , this algorithm limits certain types of data. Absolute surface areas are thus better suited for samples with regular feature frequencies. Surface area measurements on materials of suitable comparison for use in this study were absent from the literature.

1.7.2. AFM Force Measurements. Surface adhesion and frictional force measurements taken on materials similar to those used in this research, (i.e., silica and alkyl-derivatized surfaces) are numerous compared to topographic height measurements. Reference is given to hundreds of force studies applied to various disciplines in recent review articles.^[90, 107] Reports reviewed here are useful for comparing the adhesion forces between a silica tip (air-exposed silicon nitride) and native silica, etched silica, and derivatized silica surfaces. Reference to H-terminated silica is extremely limited.

Rutland and Senden^[115] mounted a single particle of colloidal porous silica ($\approx 5 \mu\text{m}$ diam.) onto a Si_3N_4 AFM cantilever as an AFM probe using epoxy resin, in a method similar to that of Ducker et al.^[147] Force measurements were made between the colloidal probe and a silicon wafer oxidized to a high density of silanol groups using the cantilever deflection mode. The manufacturer's nominal cantilever spring constant was used, since it was not possible to determine the constant experimentally. This was taken as a systematic error that scales linearly (see equation 26); thus, the force curve profiles were not affected. Experiments were conducted in aqueous solutions (NaCl as electrolyte) of the nonionic surfactant pentakis(oxyethylene) dodecyl ether to study adsorption to silica, as used in detergents and stabilizing dispersions. Interfacial energies were calculated from the ratio of the measured force to the tip radius as a normalizing function. In the absence of surfactant, forces were in the repulsive regime of the force curve, whereas an attractive force was detected at small tip-sample distances ($\approx 4 \text{ nm}$) at low surfactant concentrations.

Veeramasuneni et al^[135] used a colloidal probe identical to that used in the study above, to probe alumina (sapphire) in an aqueous (0.1 M KCl) environment as a function of pH. Cantilever constants were calculated from SEM-measured probe dimensions and Young's modulus for silicon. The calculated k averaged value was 0.12 ± 0.02 N/m for three cantilevers, compared with the manufacturer's nominal value of 0.12 N/m for each probe. Interfacial tip-sample energies were calculated as the adhesive force ratio to the tip radius, as above. Energies increased with tip-sample separation, indicating an attractive region force profile at work up to pH 8.6, attributed to positive alumina electrostatic forces. At pH values of 10.2 and 10.8, the force curves were in the repulsive region, except for a minor attraction at very close separation (< 5 nm). This small attractive region was interpreted to be due to van der Waal's interactions at work.

The pH at the point of zero charge (pzc) of alumina was determined by CEC in the experiments above. The pzc is a parameter used in electrophoresis and CEC to describe the ability of surfaces to become positively or negatively charged in aqueous solutions. The point on a plot of interfacial energies measured by AFM vs. pH where the repulsive and attractive forces intersected was at $\text{pH} \approx 9.3$, just slightly different from the pzc, $\text{pH} \approx 9.1$, determined by CEC. Thus, AFM can be used to find the point of zero charge of mineral solids.

Characterization studies utilizing surface-tip adhesion forces have been carried out on bare mica surfaces with organosilanized Si_3N_4 AFM tips,^[122] and on organosilanized silicon surfaces with bare Si_3N_4 AFM tips.^[142] Frisbie et al^[110] and van der Vegte and Hadziioannou^[124] used methylated and carboxylated combinations of coated Si_3N_4 AFM tips and microlithograph-patterned Si[100] and mica surfaces, respectively. Surface forces measured in ethanol ranged from 0.9 nN for $\text{CH}_3\text{-CH}_3$ tip-sample interactions to 1.3 nN for COOH-COOH in reference 124, and from 2 nN for $\text{CH}_3\text{-CH}_3$ interactions to 4 nN for COOH-COOH in reference 110. These factor 2 and 3 differences illustrate the need for cautious judgement when absolute AFM measurements are carried out on amorphous or vaguely-defined surfaces. Even when well-defined systems are used, conflicting methods

for calculating cantilever spring force constants and single bond forces from limited theoretical models may introduce large sources of error. Reference 124 included frequency plots of different tip-sample combinations, which clearly showed increasing ranges of bond rupture forces without being limited to absolute values.

1.8 Statement of Research Objectives

The current research proposes to address the issue of accessing the inner surfaces of 50 μm i.d. fused silica capillary tubes with the AFM probe in contact (constant force) and intermittent contact (constant amplitude) modes. The native, etched, hydrided, and C_{18} -derivatized stages of capillary modification by the silanization-hydrosilation method are examined in these modes. Specifically, the ranges of rms roughness, surface area difference, and surface-tip forces of adhesion will be presented in the form of frequency of occurrence. Additionally, the general topographic morphology of each capillary stage will be described in terms of feature dimensions and appearance. Finally, a visual comparison of the SEM and AFM methods of imaging will be examined for like scans of the CEC tube walls at each stage of development.

II. EXPERIMENTAL

2.1. Apparatus

2.1.1. AFM Instrumentation. AFM data were collected and analyzed with a Nanoscope III Multimode AFM (Digital Instruments, Santa Barbara), equipped with a CCD camera and monitor for visual positioning of the probe, laser source beam, and sample. The method of detection is cantilever deflection of a stationary laser source onto a photodiode array. This model utilizes a stationary probe holder with piezo bimorph for generating oscillations in the cantilever for the intermittent mode. The scanner, driven in the x-, y-, and z-directions by piezoelectric crystals, is mounted beneath (see Figure 15) the probe. The entire unit is damped from mechanical vibrations by a position-sensitive base. Experiments carried out in contact with capillary surfaces utilized silicon nitride probes (Park Scientific, Santa Clara) coated with gold for reflection of the incident laser beam. Silicon nitride probe nominal force constants were 0.05 N/m and 0.10 N/m, with nominal resonance frequencies of 5 - 20 kHz. Probes used in the intermittent (Tapping™) mode were silicon (Nanoprobe from Digital Instruments, Santa Barbara), with nominal force constant ranges of 20 - 100 N/m, and resonance frequency ranges of 300 - 340 kHz.

2.1.2. AFM Computer Hardware and Software. PC-compatible computer software routines for AFM parameter settings, offline image analysis, and data storage are viewed on separate monitors, one for image viewing and one for control settings. An oscilloscope analog is viewed on the imaging screen. The captured image file on the computer used for AFM control is accessible via networked computers, although the manufacturer's software is inaccessible with common software programs. Thus, if images are desired to be saved and viewed elsewhere, the images must be saved in a text information format, which precludes access to machine parameters.

2.1.3. SEM Instrumentation. SEM images were acquired with a Hitachi S-800 Field Emission Scanning Electron Microscope (Tokyo, Japan), with a nominal magnification range of x20 - x300,000. A Hummer V® Chemical Vapor Deposition

(sputtering) apparatus with a Au/Pd target was used for SEM sample conductive coating. Images were captured with a Polaroid Land® camera onto 3 in. wide by 5 in. long photographic paper, sealed with a proprietary emulsion.

2.1.4. Capillary Modification Equipment. Heating and atmospheric conditions used in capillary modification procedures were controlled by the use of a modified gas chromatograph (Hewlett-Packard Model 5890, Mountain View, CA), as shown in Figure 16.

2.2. Materials

2.2.1. Chemicals and Supplies. Fused silica capillaries with a polyimide outer coating (Polymicro Technologies, Phoenix, AZ) had an o.d. of 375 μm and an i.d. of 50 μm . Some capillary tubes with 20 μm i.d. were imaged with SEM for illustrative purposes, but were not included in the AFM research. Sectional specimens of capillaries were mounted with Scotch™ double-coated tape (3M, St. Paul, MN). Reagents used for capillary etching, silanization, and hydrosilation are previously described.^[27] Targets used in SEM sample sputtering were primary calibration standard alloys of gold and palladium.^[79] SEM mounts utilized double-coated graphitic tape (3M, St. Paul, MN). On site pre-purified liquid nitrogen was used for chilling the capillary tubes during sample preparation.

2.3. Derivatization of Capillaries

2.3.1. Etching, Silanization, and Hydrosilation. The inner bores of the capillaries were etched and chemically modified according to a previously described procedure.^[27, 29, 74] Briefly, etching proceeded by filling the capillaries with a saturated solution of ammonium hydrogen difluoride in methanol. After removing the solvent with nitrogen gas, the capillaries were heated in a modified GC oven for 2 h at 300 °C and 1 h at 400 °C in a nitrogen atmosphere, followed by a methanol rinse, and dried with nitrogen. The hydrided capillaries were prepared by flowing 1M triethoxysilane (TES) in dioxane

through etched capillaries under nitrogen pressure for 1.5 h at 90 °C, then rinsing with dioxane and toluene. Alkylation proceeded by flowing 1-octadecene with a catalyst (10 mM hexachloroplatinic acid in isopropanol) through the silicon hydride-modified capillaries under nitrogen pressure for 5 days in the GC oven at 100 °C. The alkylated capillaries were then rinsed with toluene, dioxane, and methanol, then dried with nitrogen.

2.4. Preparation of Capillaries for AFM and SEM Imaging

2.4.1. AFM Sample Preparation. The capillaries were dipped in liquid nitrogen to facilitate removal of the polyimide coating with a tempered metal razor blade. In order to expose the bores for imaging, sections of the unsheathed, chilled capillaries were then tapped with a clean aluminum tool handle to split them open. Longitudinally intact sections ($\approx 2 - 3$ mm long) were mounted under an optical microscope onto 1 cm metal discs with double-sided tape for AFM analysis. Native (bare silica) capillaries were heated in an inert atmosphere for 24 h at 106 °C, and inspected with an optical microscope for residual methanol (pretreatment solvent) prior to AFM analysis. This procedure reduced silica surface corrosion to a visually undetectable level in AFM topographic height images.

2.4.2. SEM Sample Preparation. Unsheathed capillary sections were prepared for SEM analysis by slicing transverse sections ($\approx 1 - 2$ mm long) using a tempered razor blade, and mounting on 2.5 cm discs with graphite tape, similar to the section shown in Figure 17. Longitudinal sections were also prepared for AFM comparison. The entire disc with sample sections was then sputtered under vacuum with Au/Pd in an Ar plasma to a thickness ≤ 50 Å.

2.5. AFM Procedures

AFM images were collected in the contact (constant force) mode for force-distance measurements, and in the intermittent contact (constant amplitude) mode for image height measurements in laboratory ambient conditions with relative humidity generally held to 60 - 65%.^[113, 145]

2.5.1. Intermittent Mode AFM Method. Samples were probed in the intermittent mode with the silicon cantilevers described above. After coarse positioning of the sample with the scanner, optical alignment of the incident laser beam and cantilever was optimized. Cantilever tuning was carried out such that the oscillation (drive) frequency was $\approx 85\%$ of the natural resonance frequency of the cantilever. Initial AFM settings of scan size ($3\ \mu\text{m}$), number of samples (256 pixels), upper and lower z-scan voltage limits (+220 to - 220 V), drive amplitude (30 mV), set point voltage (1 V), x,y-scan rate ($< 1 - 2\ \text{Hz}$), and the integral and proportional gains (1.0 and 5.0) for intermittent mode imaging were entered. After tip-sample contact was established, fine tuning was performed to ensure continuous tip-sample engagement during imaging. This typically entailed slightly increasing the cantilever drive amplitude. Scan rates were adjusted according to scan size and number of measured samples to provide consistent data acquisition rates.^[89] A rigorous examination of scan rate and theoretical scan speed limits has been reported by Simpson et al.^[145] The x- and y-direction scan sizes ranged from $0.5\ \mu\text{m}$ for higher resolution work, to $10\ \mu\text{m}$ for initial surveys. Optimal scan sizes for artifact-free height images were 1 and $3\ \mu\text{m}$. Images were rendered with z-height scales optimized for each surface in order to best resolve surface features. Image z-heights were rendered with the gray scale convention of dark-to-light for low-to-high surface features.^[89]

2.5.2. Contact Mode AFM Method. Contact mode imaging was performed using triangular silicon nitride cantilevers described earlier, as in Figure 10. Force-distance curves were collected every $0.25 - 0.50\ \mu\text{m}$ in various areas over a total scan window of approximately $10\ \mu\text{m} \times 10\ \mu\text{m}$ per capillary section analyzed. Several areas within the scan window were visually imaged in the contact mode to compare with force curves, in the event of unusual curve profiles. Contact mode experiments began with coarse sample-probe positioning and maximizing the SUM photodiode output (-2 V) via optical alignment. Initial AFM settings of scan size ($3\ \mu\text{m}$), number of samples (512 pixels), upper and lower z-scan voltage limits (+220 to - 220 V), set point voltage (0.0 V), and the x,y-scan rate ($< 1 - 10\ \text{Hz}$) and integral and proportional gains (5.0 and 10.0) for contact mode imaging

were entered. Note that a greater number of data samples (512 x 512) was required for contact work to produce images with good resolution. Also notable are the set point value and the lack of oscillation parameters compared with the intermittent mode. The set point voltage was typically lowered to a negative value after contact was made on rougher surfaces.

2.5.3. AFM Force-Distance Curve Acquisition. Force-distance curve measurements are taken in a “Force Calibration” mode,^[89] and the x-, y-scan rate is zero during data acquisition. An example of a force-distance curve acquired from the AFM probe tip-sample interaction on a native silica capillary surface is shown in Figure 18. The force of adhesion was calculated by the method using equation 26, $F = k\Delta x$, where F is the tip-sample adhesive force, k is the nominal (manufacturer’s reported) cantilever spring force constant, and Δx is the displacement of the cantilever during the retraction cycle of the force curve. Specifically, Δx was taken as the difference along the z-axis of the z-piezo position from the point of maximum cantilever distension (due to adhesive tip-sample forces) to its free air equilibrium position. Typical displacements ranged from ≈ 35 nm on the native silica to over 600 nm on the etched or hydrided silica. Only two cantilevers were used for all four capillary developmental stages to minimize spring force constant effects in the force-distance curve acquisitions. The first cantilever (nominal $k = 0.05$ N/m) broke during the measurement of the etched surfaces. Forces were calculated from data using the second cantilever (nominal $k = 0.10$ N/m) on the same etched and native surface, to insure that variance was minimized. Force calculations revealed no significant change in the range of forces on either surface; thus, forces measured by either cantilever are believed to be valid within the uncertainty of the nominal k values.

2.5.4. Surface Area and Surface Roughness Measurement. AFM image analyses were performed using the manufacturer’s off-line software. Specifically, rms roughness and surface area difference of capillary bore surfaces representing each derivatization step were determined according to the manufacturer’s use of published algorithms, as discussed in the Introduction.^[89] Cross-section analyses were used to

compare the size and morphology of individual features in the four derivatization steps, and to determine the extent of image flattening required for the image roughness and surface area analysis. An example of a section analysis of a native silica capillary surface is shown in Figure 19. The roughness and surface area analyses were carried out on images collected in the intermittent contact mode.

Caution is necessary in the interpretation of absolute roughness and surface area values. Unless the AFM probe tip geometry is exactly known during every image scan,^[145, 148] and piezo calibration is verified between scans,^[93] indeterminate (random) error may influence the roughness values. Thus, trends based on histogram frequencies are preferred to absolute values when reporting statistical data. All AFM images were flattened with a zero-order algorithm prior to any height analyses,^[119, 145] to correct for piezo-derived differences between scan lines. The rougher sample images (i.e., etched or hydrided) were flattened with higher order algorithms to remove image plane tilt or convolution that might result from z-piezo drift, as observed in cross-section analysis.^[145, 149] As a control, flat silicon wafers with octadecyl-derivatized surfaces were prepared to check the effect of the capillary bore curvature on the piezo motion. The topography on the silicon wafers, shown in Figure 20, and the measured surface roughnesses are in excellent agreement with those on the capillary surfaces.

2.6 SEM Procedures

2.6.1. SEM General Procedure. Samples from the same sections of capillaries to be analyzed by AFM were sputtered and loaded into the SEM sample chamber immediately after mounting. The SEM images used for direct comparison with those analyzed in the AFM mode were captured at magnifications (mostly x20,000), which could be cropped to produce scan sizes corresponding to a 3 μm x 3 μm square scan on the AFM. Higher magnifications were used for specific feature analysis. A minimal layer of conductive coating was used whenever possible (≈ 50 Å thick, according to manufacturer's data for specific sputtering time and vacuum pressure). If charging (bright white regions)

was evident on the CRT scan, a higher gamma setting was employed. Samples that did not respond to increased gamma were re-sputtered accordingly. Images were obtained in a default slow scan speed, via photographic capture. Microphotographs were allowed to develop for minimum 20 s, then removed and coated with protective emulsion.

2.6.2. SEM Parameter Settings. The electron gun (excitation) voltage was set to 10 kV for all SEM images. Since small remains of silica can cause charging, the lowest possible gamma (brightness filter) setting was used, as mentioned above. The condensor lens was generally left at a medium to low default setting, and the aperture opening was set to 300 μm . Contrast was adjusted to maximize feature resolution without losing overall visual topography. The sample stage was first adjusted to gain access to regions of interest inside the capillary tube sections, then adjusted as necessary to allow the greatest focal depth and resolution. The scan rate during image capture was ≈ 0.03 Hz in the slow scan (y) direction.

III. RESULTS AND DISCUSSION

3.1 AFM and SEM Topographic Image Analysis

A comparison of the SEM and AFM images of the capillary inner bore surfaces is presented in Figure 21, revealing good visual correspondence between electron and force two-dimensional microscopy data. Additional AFM images of the four stages of capillary modification are rendered into three dimensions in Figures 22 and 23. The z-scale in Figure 23 is exaggerated to emphasize differences in height features between the four surfaces discussed here.

Resolution differences between the AFM and SEM techniques are observable in the native sample images in Figure 21. Large scale topography and overall capillary geometry are best recorded by SEM, which allows 3-D sample positioning during CRT screen scans, without removing the specimen. However, AFM images in Figures 21-23 unveil subtle features in scans of comparatively smooth native surfaces. Superior resolution of surface features in three dimensions is thus observed in AFM, due to the close approach of the probe tip. In contrast, SEM resolution of these features requires very high magnification, limiting the scan size to areas too small to observe larger surface features. Additionally, the surfaces of samples prepared by conductive vapor deposition may experience substantial infilling by the sputtered metal coatings. Root mean square roughnesses of $< 1\text{ nm}$ as measured by AFM in this research and reported in the literature,^[136, 145] are characteristic of native silica surfaces. Under optimal conditions, a conductive layer of about 0.05 nm thick is required to produce SEM secondary electrons without noise from charging. Since the microscopic surface of silica is not flat, it is reasonable to expect some metal to fill in between silanol and silanoate clusters, effectively obscuring several Ångströms of the base depths of these surface features.

The transition from the unremarkable native surface to the pitted, but fairly smooth prominences on a typical etched surface is evident in Figures 21-23. Some etched surfaces are more abrupt, with sharply edged plates extending normal to the plane of the surface.

Optical microscopy has also detected a variety of these morphologies, resulting from different etching conditions as discussed in the Introduction. Again, the overall topography of the etched capillary surfaces is best portrayed by the SEM technique.

Further roughening and abrupt plates and holes characterize the hydrided surface in Figures 21-23. The rugged nature of the hydrided surface suggests that more than a single layer of silane may have been deposited on the etched precursor surface. Ando et al^[137, 144] produced AFM images of H-terminated silica oxidized from Si[111] surfaces with feature morphology (i.e., polygonal steps with holes) very similar to the hydrided surfaces examined in this study. The surfaces on Si[111] were produced by successive monolayer-high terraces forming one above another, as determined by AFM and ESTM analysis in fluid cells. Since the samples (highly B-doped p-type Si[111] wafers) were freshly cleaned and H-terminated by NH_4F in acidic solution immediately, the surfaces studied were fairly flat, with silica from natural corrosion presumably limited to the immediate surface. The monolayer terraces were fronted by a scallop shape, with each successive layer roughly following the shape of the preceding layer front. Another important feature noted was the distribution of holes that formed in the steps, limited to one step deep, with an approximately triangular shape in many instances. When the wafers were subjected to dilute sodium hydroxide as part of the cleaning process, the steps were etched via lateral edge destruction, and by formation of new holes. It was evident from the AFM images that a more random etching process occurred in the presence of NaOH. The results of terracing and edge and hole etching processes found above are similar to many of the features present on the hydrided surfaces found on silane-adlayered surfaces of the OTCEC capillaries studied here. The SEM image of a hydrided surface in Figure 21 infers the presence of multilayered steps with holes similar to those discussed above. When the orientation of surfaces present on the walls of etched tubes are considered, the formation mechanisms discussed above can account for the majority of the features observed on hydrided capillary surfaces. However, these features are not observed on the capillary tubes that were etched without further modification. This is interpreted to mean that either

the etched surfaces are subjected to atmospheric effects, thus destroying the initial structure, or the formation of surface silane is responsible for the step and hole structures. In the study above, H-termination occurred by reacting acid-treated Si[111] with ammonium fluoride. In the silanization-hydrosilation method, the etched surfaces are intentionally treated with NaOH to maximize the number of silanol sites. Also, silane is formed by a complete adlayer, as opposed to H-termination of existing silicon atoms. A model of silanization occurring as shown in equation 16 provides several routes for various geometries. The outcome of the surface morphology partially depends on whether the group Y in the surface product $\text{Si-O-Si(OY)}_2\text{H}$ is a predicted adjacent silane surface group, the H atom of a hydroxyl group, or a dangling silanoate bond.

Polymerization of the silanization reagent might also explain the hydrided rough surface structure. Small quantities of water molecules can initiate this polymerization, accelerated by the heat of the reaction environment (GC oven). Fairly complete polymerization of the TES has been observed in some capillaries, which caused irreversible blockage. A limited polymerization terminated by the formation of sufficient surface silane groups is consistent with the complex, layered structures observed in the AFM data discussed below.

The alkylated surface images in Figures 21-23 show that considerable filling in and smoothing of the surface occurs during the hydrosilation process. Possible remnants of pits and depressions from the etched and hydrided surfaces are visible in the SEM and AFM images. It is not possible to confirm whether the sharply defined pits and holes are attributable to pre-hydrosilation structures, or to the alkylation process from visual data alone. However, very large structures are often visible under the organic layer, which are difficult to attribute to any process other than radial plate extension and large fold formation during the etching process. Surface roughness and tip-sample force measurements, discussed later, indicate that the topographic variation, and the chemical nature of the surface are fairly uniform.

3.1.1. Native Surface Topographic Description. The surface of unreacted silica is mostly unremarkable to a horizontal resolution of ≈ 125 nm, and a vertical resolution < 5 nm. As mentioned earlier, most vertical features on native silica are ≤ 1 nm in height, although the presence of surface corrosion due to ambient humidity may produce slightly enlarged hillocks. A $5\ \mu\text{m} \times 5\ \mu\text{m}$ ($z = 6.5$ nm) intermittent contact mode AFM scan of an OTCEC tube native surface in Figure 24a shows slight undulations and surface features typically found in literature^[136, 137, 141, 142, et al] AFM images. Granular surface features < 75 nm diameter and ≈ 1 nm high characterize the overall surface. A higher resolution ($1.5\ \mu\text{m} \times 1.5\ \mu\text{m}$, $z = 3.7$ nm) intermittent contact mode AFM image in Figure 24b reveals laterally-resolved features (≈ 50 nm o.d.) with distorted hexagonal geometries, characteristic^[141] of amorphous silicon dioxide. Granular particulates (5 - 8 nm diam.) that form these hexagonal features may be clusters of silica.

Larger regions seen in the native AFM image in Figure 21 often have hillocks centered within depressions. These hillocks are believed to be nucleation sites for silica corrosion. Silanol clusters may be formed as part of a surface passivation process, typical of porous silica in ambient environments.^[24] Low surface energies as measured by AFM contact force experiments support this evidence.

The small, but deep (> 10 nm) holes in Figure 24b are not typical of native fused silica surfaces observed in this research. Therefore, these features are interpreted to be contamination defects formed during the capillary extrusion process. Medium (≈ 5 nm) depth depressions, similar to the one in the upper left corner of Figure 24b, may be regions where pit formation is initiated during the etching process.

3.1.2. Etched Surface Topographic Description. The OTCEC etched inner bore capillary surfaces are characterized by pits, grain particulates, and hillocks. Regions between these surfaces appear to be smooth, native silica. Smaller pits tend to have a circular or triangular shape, whereas medium-sized pits are usually more oblongated. Large pits can be oblong,^[137] but these structures may be described as kidney or branched shapes more frequently. The large-scale topography of a typical etched surface

is depicted in Figure 25a ($5\ \mu\text{m} \times 5\ \mu\text{m}$, $z = 300\ \text{nm}$), and in the etched surface SEM image of Figure 21. In Figure 25b, a $3\ \mu\text{m} \times 3\ \mu\text{m}$ image of the upper left region of Figure 25a shows the particulate nature of the etched capillary surface. Large kidney-shaped depressions such as the one shown in the $1\ \mu\text{m} \times 1\ \mu\text{m}$ scan of an etched surface in Figure 23 are relatively shallow ($< 100\ \text{nm}$). The $5\ \mu\text{m} \times 5\ \mu\text{m}$ etched surface in the same figure illustrates how smaller diameter holes may be quite deep ($>200\ \text{nm}$).

Regardless of the pit or depression size or shape, there are granular particles and hillocks observed around these features throughout every etched surface imaged in this research. Higher resolution contact mode AFM height images of etched surfaces in Figure 26 reveal what may be asperities formed during silica corrosion, similar to images of cleaved glass surfaces collected *in situ* under controlled corrosion conditions reported elsewhere.^[140] These asperities appear to grow in a layer above the general surface topography, upon close examination of the pits in the $1\ \mu\text{m} \times 1\ \mu\text{m}$ scans in Figure 26. Phase images (i.e., images formed by detected cantilever amplitude changes) are often useful for confirming features suspected as being separate layers or materials. Such a phase image of the same etched surface scanned in Figure 25a is shown in Figure 27. The pit regions clearly show a separate layer below the upper surface, as compared with shallow depressions. The presence of light (high) areas on the right of each pit indicates that either the sample specimen was oriented in a slight upward direction to the right, or the AFM tip was worn in such a way that the far side of each pit was encountered as a wall, in the retrace (left to right) direction of the scan. The black and white tip “chatter” in the lower right of the image is probably the result of excessive frequency drive amplitude.

The reliability of the AFM method is demonstrated by the images in Figure 26. Both images were captured as $1\ \mu\text{m} \times 1\ \mu\text{m}$ scans, but each sample is from a different capillary (etched at different times). Furthermore, the surface imaged in Figure 26a was scanned in contact mode AFM with a silicon nitride probe, and that in Figure 26b was scanned in intermittent contact mode AFM with a silicon probe. The z-heights (33.9 nm and 38.9 nm, A and B respectively) are reasonably close for feature comparison.

The microscopic mechanism of etching fused silica surfaces is difficult to describe, due to the amorphous nature of the material. However, localized phenomena may be compared with studies carried out on surfaces that are better defined. Some structures observed here hint at the geometric origins of fused porous silica or fused silica glass. Specifically, the [111] diamond lattice in pure silicon and in SiO₂ minerals such as quartz, is retained in random fashion as molten materials fuse.^[1] The orientation of atoms is more randomized in regions where the original geometry is not retained, but may be attributed in many cases to expanded or contracted ring structures. This is consistent with the AFM topographic description of the surface of fused native silica.

The presence of atomic defects in crystalline materials often accelerate the rate of etching, which can be manipulated by the chemistry of the etchant. Furthermore, the morphology of etched pits often reflects the underlying lattice geometry.^[150] In a kinetic Monte Carlo simulation of Si[111] etching, Flidr et al^[151] examined several modes of surface modification, e.g., step, pit, and miscut surface etching. Two important points in this simulation lend evidence for the nature of the etched (and possibly, the hydrided) surfaces researched here.

First, the bilayer geometry in the Si[111] crystal lattice determines the morphology of layered steps. Steps oriented in the (112) direction (first bilayer) form triangles pointing up as viewed normal to the layer, and steps oriented in the (112) direction (second bilayer) point down. These geometries are also applicable to SiO₂ crystal structures. Other shapes are formed from irregular hexagons, which are basically triangles with their points removed. The Si[100] surface develops roughly square pits, depending on the etchant. The introduction of surface defects or edge miscuts is the basis for highly irregular shapes that could be construed as chaotic in origin. The triangular, square, and modified hexagonal shapes of pits and steps are documented in the SEM and AFM images of the etched and hydrided surfaces presented here. Pits more circular in appearance may simply be squares with corner defects.

A second point of interest is the kinetic control of morphology, due to competing reactions during the etching process. When pure silicon is etched, the surface becomes H-terminated, as discussed earlier.^[137, 144] In the surface etching process,^[151] there are at least six distinct chemical sites on Si[111], giving a minimum equal number of possible rate-limiting reactions in the presence of a simple fluoride etchant. Surface steps oriented in particular directions will have different orders (i.e., mono-, di-, tri-) of surface hydrides. Each order of hydride and thus, each particular face of the crystal, will have a different etching rate. Since the face orientation and bilayer exposure determines the resulting morphology, there is a direct dependence of morphology on kinetics, i.e., features corresponding with the slowest etching reaction will reach the steady state. This is also true in pit formation predicted by the Monte Carlo simulation. For example, when a vertical dihydride site etches more slowly than its horizontal monohydride counterpart, triangular pits with SiH₂ surfaces are formed. If the rates are reversed, the triangle is flipped, and the surface is SiH-terminated. Equal rates predict a rough hexagonal shape.

3.1.3. Hydrided Surface Topographic Description. Sharper, more pronounced features describe the overall topography of hydrided surfaces, when compared with the etched and alkylated surfaces in Figure 22. The wide range of features seen in the AFM image in Figure 21 suggests that a combination of etching and regions of multiple adlayering forms the hydride surface. Extremes in vertical heights between adjacent prominences and deep pits can approach 2 μm , making AFM imaging difficult.

The shape and thickness of layered plates that crop out of the hydrided surface also appear to be nearly identical in many cases. The layers and plates were initially believed to be multiple-point tip artifacts. However, a comparison of contact and intermittent mode AFM images collected with different probes, along with higher magnification ($\geq \times 50,000$) SEM images reveals that these are authentic features. Figure 28 shows part of this SEM and AFM evidence. The plates could be attributed to vestigial close-packed [111] plane faces in crystalline silicon dioxide. This interpretation is also suggested by a SEM image of broken plates evident in an etched CEC tube, shown in Figure 29.

The mid-sized features on the hydrided surface are dominated by plateaus or steps and holes which appear to have grown in layer fashion. As seen in the SEM image in Figure 21, this growth interpretation is consistent with literature data^[144, 150] and model simulation.^[151] Plateaus and holes in the hydrided capillaries range in lateral size from < 100 nm to $> 1\mu\text{m}$. In Figure 30, a low-power SEM image ($\times 1000$) of the hydrided surface shown in Figure 28 gives a wider perspective of the step-hole layering occurring on these surfaces. The small pits (< 50 nm) observed on the etched surfaces, particularly in and around the larger, more gently sloped kidney-shaped depressions ($\approx 0.75\mu\text{m}$), may provide nucleation sites for silane adlayering.

Hydrided pits more closely resemble deep canyons and fissures, as suggested in Figure 31a. Also notable in this $5\mu\text{m} \times 2.5\mu\text{m}$ intermittent mode image is the presence of nearly duplicate layers. The rugged nature of the hydrided surface suggests that more than a single layer of silane may have been deposited on the etched precursor surface, as mentioned earlier. A limited polymerization of the silanization reagent, TES, possibly explains this phenomenon. Further evidence of a layer-by-layer formation of the silane surface is shown in the rather complex $5\mu\text{m} \times 5\mu\text{m}$ phase image in Figure 31b. Layering is evident in the parallel and normal directions, and oblique to the surface. Thin parallel lines are interpreted to be the edges of rows of stacked plates, similar to the edges of the replicate plates indicated in the image in Figure 31a. The differences between the AFM height (constant amplitude) and AFM phase (constant z-piezo voltage) methods in the intermittent mode are apparent in Figure 31. While z-piezo voltages required for shifting the cantilever to a new position in response to amplitude changes are registered in the height method (see Figure 31a), the cantilever is held at a constant height (voltage) above the surface, and changes in the phase of the detection signal are monitored as the cantilever deflects (see Figure 31b) in the phase method. The height method affords a continuum of data points during image construction. However, sharp edges and other fine features may be lost during the scan, especially if the piezo does not reposition as rapidly as the cantilever deflects. Conversely, the cantilever may experience residual overtone

oscillations,^[89] leading to false laser output data. Thus, the images of complex surfaces with extreme z-plane features need to be analyzed carefully, and very fine tuning of the cantilever is required.

3.1.4. Alkylated Surface Topographic Description. The general topography observed in the 3-D surface plot of the alkylated sample in Figure 22 indicates smoothing, and possibly some filling in of features during the hydrosilation step of capillary tube modification. The 3-D plots in Figure 23 also suggest this change in topography, when compared with the hydrided surface images. The AFM images in Figure 20 of octadecyl-modified: a) hydrided flat silicon, and b) etched, hydrided capillary inner bore surfaces reveal clusters, or possibly adjacent assemblies of the C₁₈ moieties. These clusters average 80 nm in diameter, and are visible on the surface of an earlier derivatized capillary, shown in Figure 32a. This image only hints at the complex structure of plates, pits, and holes left from the hydrided precursor of hydrosilation.

A drastic change in topography occurs when a capillary surface experiences more aggressive etching, which can remove significant tube wall volume. The surface occasionally becomes defined by large plates and escarpments, as seen in Figure 32b. Other etching effects include a capillary tube i.d. increase of up to 2 μm (see Figure 3) at the basal surface, and the presence of very large possibly detached (as determined by phase imaging), but stationary structures resting on the surface. Even these somewhat anomalous features appear to be thoroughly derivatized. This is evidenced by Figure 32b and other AFM images, as well as a fairly uniform distribution of surface forces, discussed later.

An inspection of Figures 32a and 20b suggests that more chromatographic sorption sites are formed when the capillary surface is constructed via microscopically mild pitting, and macroscopically gentle folding during the etching process. Although the presence of structures formed normal or oblique to the surface (see Figure 32b) certainly contribute increased macroscopic surface area, a lack of exposed sublayers (thus, reaction sites), is noted on these features. Sublayers with more reactive-site exposure to possible mobile phase solutes encountered in CEC are present on the surfaces in Figures 32a and 20b.

Another notable topographic feature of the etched, alkylated capillary surface is the apparent change in pit and large depression size. The larger pit size dimensions ($< 0.5 \mu\text{m}$) in the alkylated surface are somewhat smaller than those in the etched surface. The largest depression observed is about $0.8 \mu\text{m}$ long, seen in the SEM image in Figure 33b. Figure 33 compares a high magnification ($\times 120,000$) SEM image with a high resolution AFM image of the same alkylated surface. The absence of smaller features in the SEM image illustrates the superior resolution inherent in the scanning probe microscopies.

In summary, the surface topography of each of the four stages of capillary modification is fairly unique. The progression of changes is represented especially well in the SEM images of Figure 21, and the 3-D surface plots in Figures 22 and 23. The mechanisms of etching^[150, 151] and silanization^[137, 144] presented in the literature provide possible explanations for some of the structures noted here. The chemical basis for C_{18} hydrosilation (equation 17) is consistent with the interpretation of the AFM images of alkylated surfaces. These surfaces have shown enhanced electrochromatographic properties,^[27] discussed later.

3.2 AFM Surface Variation Measurements

3.2.1. RMS Roughness Measurements. The rms roughnesses of the four surfaces are in the following order: Native $<$ Etched $\approx$ Alkylated $<$ Hydrided. The frequency histograms of the rms roughnesses are plotted in Figure 34. Numerical values for roughness reveal the following: 1) most native capillaries have a roughness $< 1 \text{ nm}$, in agreement with literature AFM silica studies;^[136, 145] 2) the etched capillaries have a wide distribution of roughnesses, with subpopulations centered around 5 nm , 15 nm , and 30 nm ; 3) the hydrided capillaries also have a wide distribution, with subpopulations centered around 10 nm and 50 nm , and a maximum roughness ($\approx 80 \text{ nm}$) twice that of etched or alkylated surfaces; 4) alkylated capillaries have a roughness centered at 15 nm , similar to the lower roughness populations of the etched and hydrided capillaries. The increased roughness of the etched surfaces is predicted, but the sizable increase found

in the hydrided surfaces was unexpected. However, visual topographic evidence concurs with these observations on all of the capillary surfaces investigated here. This increase can be partially explained by prominences and pits observed in large, canyon-like regions of the silane-adlayered surface. Such a region is noted in the $1\ \mu\text{m} \times 1\ \mu\text{m}$ 3-D image of a hydrided OTCEC capillary in Figure 23. As mentioned earlier, irregular growths may result from polymerization of the silanization reagent, TES. Smoothing of the hydrided surfaces after alkylation may be due to infilling of the pits by alkyl groups.^[124]

3.2.2. Surface Area Measurements. The surface areas of the four surfaces are in the following order: Native < Etched < Alkylated < Hydrided. The ordering roughly correlates with that of the rms roughness. The frequency histograms of the surface area increases are plotted in Figure 35. The etched surface area is greater than the native surface area, as expected from the etching process. The distribution of surface areas for etched samples is explained by an image analysis (see Figures 21 and 23) of the sizes and number of pits in the etched surface. Smaller, shallower pits occur frequently, yielding a peak in the surface area difference measured in the x,y-plane, whereas less frequent larger, deeper (50 - 100 nm depth) depressions contribute a low yet noticeable number of higher surface area values. The hydrided surface AFM images rendered in 3-D in Figure 23 illustrate how a 100 nm wide pit in the etched surface would contribute less surface area and roughness than a similar width pit in the hydrided surface. The hydrided surfaces have deeper (minimum 100 - 250 nm depth) abrupt holes that contribute to high rms roughness values and high surface areas. Canyon-like depressions in the hydrided surfaces are built up from layers of plates > $1\ \mu\text{m}$ in depth. Small holes in the plates further contribute to the roughness. This combination of large and small scale features explains a wide distribution of roughness and surface area values. As seen in the images in Figures 21 and 22, the alkylated surfaces have more regular features overall than the precursor hydrided surfaces. This is evidenced by lower surface area histogram frequencies seen in Figure 35.

3.3 Surface Force Measurements

3.3.1. Surface Force Distributions. The relative magnitude of the surface-tip forces of attraction for the four surfaces is in the following order: Native $\approx$ Alkylated $\approx$ Hydrided I < Etched < Hydrided II. The frequency histograms of the surface forces are plotted in Figure 36. Hydrided surfaces have two distinct populations of forces, hence the assignments I and II in the ordering scheme. The ordering of surface forces can be qualitatively explained by the relative reactivities of the four surfaces as follows.

The native surface has been exposed to atmospheric conditions long enough to arrive at a fairly low surface free energy. Thus, minimization of the attractive force of adhesion between the silicon nitride probe tip and the surface is observed. This passivation effect is similar to that experienced by porous silica exposed to ambient humidity, and is consistent with tip-sample interaction theory. Ito et al^[122] measured surface forces on freshly-cleaved mica and hydroxyl-terminated monolayers on mica with an unmodified silicon nitride AFM tip. Higher forces observed on the freshly-cleaved samples were attributed to (electrostatic) charge-dipole interactions. The interactions arise from the negative surface charge on the fresh SiO₂ surface of mica (in unbuffered water), and silanol groups on the Si₃N₄ tip. Lower forces observed on the hydroxyl-terminated surfaces are attributed to the similar nature of the hydrophilic sample and tip surfaces, with low surface free energies. These observations are reflected in the distribution of forces on etched capillary surfaces, discussed below. Reduced forces between like surfaces are also observed between hydrophobic materials used on derivatized tips and samples, e.g., CH₃-CH₃, attributed strictly to van der Waal's interactions.^[122]

The etched surfaces on OTCEC capillary bores have become more active from increased silica being exposed to the AFM tip via a maximized surface area. The activated regions may be protected from ambient moisture prior to AFM sample preparation, evidenced by the presence of high surface force populations in the etched histogram. Silica surfaces exposed to ambient humidity experience a general passivation through the corrosion process, depending on the localized pH.^[113] These surfaces are expected to

display minimized tip-sample interactions, similar to those measured on the native silica capillary walls. Thus, regions with high surface forces (hence, high surface free energies) are interpreted to represent activated, relatively corrosion-free areas. Additionally, possible electrostatic contaminants in the fluid air layer, and the capillary action of the fluid layer, may yield a shift toward higher attractive forces. Lower force populations on the etched surface appear to coincide with passivated (i.e., ambient-corroded) regions similar to those on native silica surfaces. Another possible contribution to the wide distribution of forces on etched surfaces is the dependence of potential energy on the tip-sample separation distance as derived^[94] in equation 24, and in Johnson-Kendall-Roberts^[152] (JKR) theory. These equations do not account for any additional forces that may work on the sides of the tip apex. Thus, a spectrum of forces oblique and parallel to the surface may also act on the tip, due to pits and ledges encountered on etched surfaces.

From the relative ordering of the surface forces of the two hydrided populations, it could be concluded that the hydrided II population represents the actual hydride adlayer species, while the hydrided I population is unreacted silica, i.e., not silane-adlayered. The assignment of unreacted native silica to the lower force hydrided I population is consistent with DRIFT and NMR results mentioned earlier.^[19] With these assignments, hydrided populations I and II would be interpreted as passivated and activated surfaces, respectively. Although the number of force investigations using organosilane-adlayered AFM tips and samples is vast,^[107] the specific measurement of forces on H-terminated silicon (silane) was not reported in the literature.

The tip-sample attractive region forces of the alkylated surfaces were comparable to the native and hydrided I force populations. The potential energy of an alkylated surface may be viewed as being lowered (passivated) with respect to an AFM tip due to the hydrophobic nature of the hydrocarbons substituted on the hydrided surface. This is consistent with low hydrophilic tip-hydrophobic sample interfacial energies observed on alkane surfaces,^[122-124] and relatively low surface free energies associated with an alkyl-terminated (e.g., CH₃) surface.^[124] Rutland and Senden^[115] observed lower cantilever

pull-off forces on organic-derivatized (i.e., hexakis(oxyethylene) dodecyl ether) quartz surfaces for a given tip-sample distance, than with unmodified oxidized Si wafer surfaces, using a porous silica-modified AFM cantilever. In the investigation of the effects of mica surface and silicon nitride tip modifications measured by AFM forces, Ito et al^[122] found that surface-tip forces of vinyl-terminated layers on mica were minimized compared to those on the freshly-cleaved mica (SiO₂) mentioned above. These results are consistent with differences in forces between the alkylated and etched surface forces observed in Figure 36, supporting the interpretation of a complete hydrosilation of these surfaces.

3.3.2. Force-Distance Curve Profiles. The majority of the observed force curves had vertical (near zero cantilever displacement) regions of tip release from the sample, indicating that adhesion was due to tip-sample forces of attraction, as opposed to capillary forces from surface contaminants.^[122-124] However, the etched capillaries had tip-sample release cycles (see Figure 14, release at position 7 to free air equilibrium) that deviated from the vertical plane, and from linearity. This deviation can be attributed to a surface covered with an ambient moisture layer, resulting in capillary forces. Another source of non-linear behavior between the tip and sample could be additional forces of attraction due to lateral interactions between the porous-like etched surface and the sides of the tip. Further evidence in the form of approach-cycle attractive region cantilever deflection (i.e., tip snap), occurring in several of the etched force curves supports the capillary force interpretation. Thus, the high force values observed in the etched capillary frequency plot (see Figure 36) may be heavily weighted by fluid film effects.

It is possible to acquire a force curve at a specific site on a given surface.^[89] When a contact mode AFM tip is engaged with the surface, the x,y-position is stored as an image parameter. The instrument responds to a force curve acquisition command by positioning the tip in the center of the current scan, and samples the surface. Thus, the force curves of specifically passivated and activated sites on an etched surface provide evidence supporting the interpretation of the distribution of etched surface forces. The images and force curves in Figures 37 and 38 were captured using the same AFM probe and machine parameters on

the same etched specimen, giving a reasonably reliable assessment of chemical information via forces associated with specific surface features. In Figure 37a, a force curve is captured on a surface region (Figure 37b), believed to be the product of a silica corrosion process influenced by ambient humidity, as discussed in the topographic description of etched surfaces. The calculated force in this region is ≈ 5 nN, roughly midway between hydrogen bonding (dissociation energy ≈ 20 kJ/mol)^[17, 91] and van der Waal's interactions (dissociation energy ≈ 2 kJ/mol). Thermodynamic considerations predict a passivated surface in this environment, leading to lowered surface interaction energies with a similarly exposed AFM tip.^[107] A more protected area, i.e., below the boundary of a pit, is probed on an etched surface in the image shown in Figure 38b. In Figure 38a, the presence of a very strong tip snap infers that capillary and/or electrostatic forces may be at work between the sample and tip. Also, the shape of the curve during the tip release is indicative of long-range, lingering tip-sample interactions. These observations are consistent with a high surface free energy, which correlates with high tip-sample adhesive force.^[124] The calculated force in this region is ≈ 25 nN, comparable to hydrogen bonding.^[80, 91] Note the smaller overall curve in Figure 37a, and minimal tip snap (the abscissae and ordinates of the force plots in Figures 37 and 38 scale equally). Since these measurements were made in the laboratory atmosphere (humidity 60 - 65 %), a force contribution from the fluid air layer is consistent with the values mentioned. The magnitudes of tip-sample adhesive forces decrease when the experiments are carried out in a fluid cell environment.^[107 et al]

The hydrided II release section of the force curve retraction cycle also deviated from the vertical plane, but remained linear. A minimal tip snap during the tip-sample approach cycle into the repulsive region of the force curve was also observed. Thus, the high attractive region forces observed in the hydrided II population are believed to be real (non-capillary) interactions, possibly due to long range electrostatic and hydrogen bonding attractions. The native and alkylated surfaces did not show hydration effects in the force curve profiles.

3.3.3. Further Force Studies. Since force measurements are subject to many influences as discussed above, further studies may include derivatizing cantilever tips, using the same synthetic pathways as those reactions used for the hydrosilation of etched, fused silica capillary tube walls. Derivatized sample surfaces with solute-bonded phases might be probed with derivatized tips in a fluid cell tailored to a desired mobile phase, thereby modeling a nearly complete CEC experiment at the microscopic level. Another group of experiments could probe capillary surfaces in the presence of surface potential, to model electrophoretic (e.g., double layer formation) behavior.

3.4 Comparison of AFM Surface Roughness, Area, and Force Results

The results from the measurement of roughness, surface area, and surface-tip forces are summarized in Table 1. It is reasonable to question whether measured surface forces are influenced by the surface areas observed here. Adhesive forces do theoretically depend on a classical contact area treatment between a probe tip and sample. The JKR theory predicts a direct dependence of the force of adhesion on the radius of the AFM tip.^[152] However, large differences in surface roughness and surface area distributions between the native and alkylated surfaces as observed in Figures 34 and 35, and Table 1, do not correlate with the native and alkylated surface force results.

Although AFM image height measurements were carried out in the intermittent contact mode, similar results were obtained from analysis of images collected in the contact mode. Contact mode topographic visual images agree with those in the intermittent contact mode in this research. The agreement between data collected in these modes initially appeared to contradict other research^[145] carried out on very smooth and mildly roughened surfaces, i.e., mica for smooth surface analyses and quartz for rough analyses. A significant variation in roughness may be attributed to a non-linear piezo response^[89, 90] and to tip or sample modification during scanning, particularly in the contact mode. Sample material transfer to the tip has been documented.^[107, 153] Another study^[113] reports that soft, loose particles resulting from the oxidative corrosion of potash-lime-silica glass can be

moved by the AFM tip in the contact mode. The above study also points out that the oscillating tip in the intermittent contact mode may activate surface processes via energy transfer to the sample surface, as evidenced by an increased *in situ* corrosion rate observed on the glass surfaces. Thus, careful control over the parameters of data acquisition rate and applied force is required in either AFM mode. The native silica roughnesses measured on the OTCEC tube walls in the research here (contact and intermittent contact modes: 0.8 +/- 0.3 nm) are within the range of experimental error (0.8 +/- 0.7 nm for contact mode, and 1.2 +/- 1.2 nm for intermittent contact mode) of published quartz surface data.^[145]

3.5 Correlation of Surface Morphology to Chromatographic Behavior

The electrokinetic chromatographic behavior of the etched chemically modified capillaries has been studied for a number of different solutes.^[71] A tentative explanation for the retention and adsorption properties of the capillaries is supported by the AFM measurements reported here. As mentioned in the Introduction, OTCEC experiments that examine the behavior of basic compounds in capillaries modified to provide stationary phase material on the tube walls are of particular interest. Small amine, peptide, and protein solutes often show electrochromatogram peak tailing due to strong interactions with silanol groups present on (native) underivatized capillary surfaces. The AFM and SEM visual images and surface morphology measurements characterize the etched, chemically modified OTCEC tube walls as being completely coated, particularly after the organic moiety has been bonded via hydrosilation. The OTCEC behavior of basic solutes on etched chemically modified capillaries follows.

Capillary electrochromatogram peaks using an etched, C₁₈-modified OTCEC tube are very symmetrical when compared to peaks observed from a bare uncoated capillary, indicating minimal interaction with silanol groups.^[27, 71] The elution time for these basic solutes in the etched C₁₈-modified tube is significantly longer than the time observed using unetched C₁₈-modified tubes. This behavior indicates that solute-bonded phase interactions

are clearly part of a chromatographic process in the elution mechanism of OTCEC using etched C₁₈-modified tubes prepared by the silanization-hydrosilation method. Solute peak widths observed using etched, chemically modified tubes are larger than those for solutes which migrate through a native fused silica capillary. This behavior provides further evidence of the presence of increased solute-bonded phase interaction, since peak broadening occurs only as a result of mass transfer effects, discussed in the Introduction.^[7] The elution times of solutes are also longer as the diameter of the capillary is diminished. This may be explained by larger retention factors (k' values) that result from a shorter solute diffusion distance required for interaction with the bonded organic moiety.^[154]

IV. CONCLUSION

Samples representing the four stages of capillary modification have been prepared and measured by AFM for roughness, surface area, and surface-tip interactive forces. Agreement between SEM and AFM topographic images is good. AFM measurements contribute additional 3-D information through three-dimensionally rendered surface plot images, surface areas, and roughness. These measurements and the surface adhesion force results contribute new characterizational parameters to the study of chemically modified OTCEC capillary tube wall configurations.

The surface roughnesses and surface areas of the four stages of modification are in the following order: Native < Etched $\approx$ Alkylated < Hydrided. This order is explained on the basis of a series of steps starting with a native oxide, proceeding through surface roughening, and ending with the addition of silyl and alkyl groups. The tip-sample interaction forces of the four stages are in the following order: Native $\approx$ Alkylated $\approx$ Hydrided I < Etched < Hydrided II. Hydrided I and II represent two subpopulations within the hydrided surface force data. The ordering of forces is explained by the ordering of the surface activity of the chemical species that result from each step in the derivatization process. The native, alkylated, and hydrided (I) capillary bores are characterized by low surface energy, passivated surfaces, displaying weak interaction with the AFM probe tip. The etched and hydrided (II) capillary bores are characterized by high surface energy, activated surfaces, displaying strong interaction with the AFM probe tip. This AFM characterization of surface passivation is demonstrated to be consistent with the chromatographic behavior of these derivatized CEC columns, particularly toward basic solutes. Thus, even partially reacted hydride surfaces detected by NMR, DRIFT, and AFM force studies appear to provide a suitably activated precursor, leading to completely alkylated OTCEC column tubes.

REFERENCES

1. Brown, T.L.; LeMay, Jr., H.E.; Chemistry, The Central Science, Prentice-Hall, Englewood Cliffs, N.J., 1985.
2. Cassidy, H.G.; Fundamentals of Chromatography, Wiley-Interscience, New York, 1957.
3. Heftmann, E., Ed.; Chromatography: A Laboratory Handbook of Chromatographic and Electrophoretic Methods, Van Nostrand Reinhold, New York, 1995.
4. VanHall, C.E., Ed.; Modern Methods of Analytical Chemistry, Dow Chemical, Midland, MI., 1985.
5. Giddings, J.C.; Dynamics of Chromatography, Part I, Principles and Theory, Marcel Dekker, Inc., New York, 1965.
6. Fischer, L.; An Introduction to Gel Chromatography, North-Holland, London, 1969.
7. Giddings, J.C.; J. Chem. Ed., 44 (1967) 704.
8. Vansant, E.F., Van Der Voort, P., Vrancken, K.C.; Characterization and Chemical Modification of Silica, Elsevier, Amsterdam, 1995.
9. Lee, M.L., Yang, F.J., Bartle, K.D.; Open Tubular Column GC; Wiley, New York, 1984.
10. Schomburg, G.; Gas Chromatography; VCH, Weinheim, Germany, 1990.
11. Camilleri, P.; Capillary Electrophoresis; CRC Press, New York, 1998.
12. Colon, L.A., Guo, Y., Fermier, A.; Anal. Chem., 69 (1997) 461A.
13. Heftmann, E., Ed.; Chromatography: A Laboratory Handbook of Chromatographic and Electrophoretic Methods, Van Nostrand Reinhold, New York, 1975.
14. Mikkers, F.E.P., Everaerts, F.M., Verheggen, Th.P.E.M.; J. Chromatogr., 169 (1979) 11.
15. Grushka, E., McCormick, R.M., Kirkland, J.J.; Anal. Chem., 61 (1989) 241.
16. Jorgenson, J.W., Lukacs, K.D.; Anal. Chem., 53 (1981) 1298.
17. Linde, D.R., Ed., Handbook of Chemistry and Physics, CRC Press, Boca Raton, FLA., 1991.
18. Jorgenson, J.W., Lukacs, K.D.; Science, 222 (1983) 266.

19. Pesek, J.J., Sandoval, J.E., Su, M.; *J. Chromatogr.*, 630 (1993) 95.
20. Pesek, J.J., Matyska, M.T., Hemphala, H., Christensen, P.; *J. Liq. Chromatogr.*, 18 (1995) 2507.
21. Smith, N.W., Evans, M.B.; *Chromatographia*, 38 (1994) 649.
22. Pesek, J.J., Matyska, M.T., Sandoval, J.E., Williamsen, E.J.; *J. Liq. Chrom. & Rel. Technol.*, 19 (1996) 2843.
23. Pesek, J.J., Tang, V.H.; *Chromatographia*, 39 (1994) 649.
24. Unger, K.K.; *Porous Silica - Its Properties and Use as Support in Column Liquid Chromatography*, Journal of Chromatography Library, Vol. 16, Elsevier, Amsterdam, 1979.
25. Iler, R.K.; *The Chemistry of Silica*, Wiley, New York, 1979.
26. Knox, J.H., Pryde, A.; *J. Chromatogr.*, 112 (1975) 171.
27. Pesek, J.J., Matyska, M.T.; *J. Chromatogr. A*, 736 (1996) 255.
28. Tiselius, A.; *Trans. Faraday Soc.*, 33 (1937) 524.
29. Pesek, J.J., Matyska, M.T.; *J. Chromatogr. A*, 736 (1996) 313.
30. McLaughlin, G.M., Nolan, J.A., Lindahl, J.L., Palmieri, R.H., Anderson, K.W., Morris, S.C., Morrison, J.A., Bronzert, T.J.; *J. Liq. Chromatogr.*, 15 (1992) 961.
31. Lukacs, K.D., Jorgenson, J.W.; *J. High Res. Chromatogr.*, 8 (1985) 407.
32. Bruin, G.J.M., Chang, J.P., Kuhlman, R.H., Zegers, K., Kraak, J.C., Poppe, H.; *J. Chromatogr.*, 471 (1989) 429.
33. VanOrman, B.B., Liversidge, G.G., McIntire, G.L., Olefirowicz, T.M., Ewing, A.G.; *J. Micocol. Sep.*, 2 (1990) 176.
34. Pretorius, V., Hopkins, B.J., Schieke, J.D.; *J. Chromatogr.*, 99 (1974) 23.
35. Jorgenson, J.W., Lukacs, K.D.; *J. Chromatogr.*, 218 (1981) 209.
36. Terabe, S., Otsuka, K., Ando, T.; *Anal. Chem.*, 57 (1985) 834.
37. Terabe, S., Otsuka, K., Ichikawa, K., Tsuchiya, A., Ando, T.; *Anal. Chem.*, 56 (1984) 111.
38. Knox, J.H., Grant, I.H.; *Chromatographia*, 24 (1981) 209.
39. Yan, C., Schaufelberger, D., Erni, F.; *J. Chromatogr. A*, 670 (1994) 15.

40. Knox, J.H., Grant, I.H.; *Chromatographia*, 32 (1991) 317.
41. Rebscher, H., Pyell, U.; *Chromatographia*, 38 (1994) 737.
42. Kitigawa, S., Tsuda, T.; *J. Micocol. Sep.*, 6 (1994) 91.
43. Yang, F.; *J. Chromatogr.*, 236 (1982) 265.
44. Ishii, T., Takeuchi, T.; *J. Chromatogr.*, 255 (1983) 349.
45. Alborn, H., Stenhagen, G.; *J. Chromatogr.*, 323 (1985) 47.
46. Yamamoto, H., Baumann, J., Erni, F.; *J. Chromatogr.*, 593 (1992) 313.
47. Tsuda, T., Tanaka, I., Nakagawa, G.; *Anal. Chem.*, 56 (1984) 1249.
48. Vindevogel, J., Sandra, P.; *Electrophoresis*, 15 (1994) 842.
49. Martin, M., Guiochon, G.; *Anal. Chem.*, 56 (1984) 614.
50. Martin, M., Guiochon, G., Walbroehl, Y., Jorgenson, J.W.; *Anal. Chem.*, 57 (1985) 559.
51. Onuska, F., Comba, M.E., Bistricki, T., Wilkinson, R.J.; *J. Chromatogr.*, 142 (1977) 117.
52. Sandoval, J.E., Pesek, J.J.; *Anal. Chem.*, 61 (1989) 2067.
53. Sandoval, J.E., Pesek, J.J.; *Anal. Chem.*, 63 (1991) 2634.
54. Chu, C.-H., Jonsson, E., Auvinen, M., Pesek, J.J., Sandoval, J.E.; *Anal. Chem.*, 65 (1993) 808.
55. Montes, M.C., van Amen, C., Pesek, J.J., Sandoval, J.E.; *J. Chromatogr. A*, 688 (1994) 31.
56. Sandoval, J.E., Pesek, J.J.; US Pat. 5 017 540 (1991).
57. Sandoval, J.E., Pesek, J.J.; US Pat. 5 326 738 (1994).
58. Huber, J.F.K., Kolder, F.F.M., Miller, J.M.; *Anal. Chem.*, 44 (1972) 105.
59. Wirth, M.J., Fatunmbi, H.O.; *Anal. Chem.*, 65 (1993) 822.
60. Pesek, J.J., Swedberg, S.A.; *J. Chromatogr.*, 361 (1986) 83.
61. Bowser, J.R.; *Inorganic Chemistry*, Brooks/Cole, Pacific Grove, California, 1993.
62. Morrison, R.T., Boyd, R.N.; *Organic Chemistry*, Allyn and Bacon, Boston, 1974.

63. Pesek, J.J., Matyska, M.T.; *J. Chromatogr.*, **687** (1994) 33.
64. Pesek, J.J., Matyska, M.T., Soczewinski, E., Christensen, P.; *Chromatographia*, **39** (1994) 520.
65. Pesek, J.J., Lu, Y., Siouffi, A.M., Grandperrin, F.; *Chromatographia*, **31** (1991) 147.
66. Saito, Y., Jinno, K., Pesek, J.J., Chen, Y.L., Luehr, G., Archer, J., Fetzer, J.C., Biggs, W.R.; *Chromatographia*, **38** (1994) 295.
67. Saito, Y., Ohta, H., Nagashima, H., Itoh, K., Jinno, K., Pesek, J.J.; *J. Microcol. Sep.*, **7** (1995) 41.
68. Pesek, J.J., Matyska, M.T., Williamsen, E.J., Tam, R.; *Chromatographia*, **41** (1995) 507.
69. Ohta, H., Saito, Y., Jinno, K., Pesek, J.J., Matyska, M.T., Chen, Y.L., Archer, J., Fetzer, J.C., Biggs, W.R.; *Chromatographia*, **40** (1995) 507.
70. Chiari, M., Nesi, M., Sandoval, J.E., Pesek, J.J.; *J. Chromatogr. A*, **717** (1995) 1.
71. Matyska, M.T., Pesek, J.J., Katrekar, A.; *Anal. Chem.*, In Press.
72. Vickerman, J.C., Ed.; *Surface Analysis, The Principle Techniques*, Wiley, Chichester, 1998.
73. Pesek, J.J., Sandoval, J.E., Williamsen, E.J.; *Trends in Appl. Spectroscopy*, **1** (1993) 41.
74. Pesek, J.J., Matyska, M.T., Mauskar, L.; *J. Chromatogr. A*, **763** (1997) 307.
75. *S-800 Field Emission Scanning Electron Microscope, Instruction Manual*, Hitachi Instruments, 1993.
76. Albrecht, T.R.; Ph.D. Thesis, Stanford University, June 1989.
77. Binnig, G., Quate, C.F., Gerber, Ch.; *Phys. Rev. Lett.*, **56** (1986) 930.
78. Hollas, J.M.; *Modern Spectroscopy*, Wiley, Chichester, 1992.
79. Deline, V.; IBM Almaden Research Center, San Jose, CA, personal communication, 1996.
80. Alberty, R.A.; *Physical Chemistry*, Wiley, New York, 1983.
81. Sanders, J., Hunter, B.K.; *Modern NMR Spectroscopy*, Oxford University Press, Oxford, 1990.

82. Frederickson, L.D.; *Anal. Chem.*, 26 (1954) 1883.
83. Peri, J.B.; *J. Phys. Chem.*, 69 (1965) 211.
84. Kohler, J., Chase, D.B., Farlee, R.D., Vega, A.J., Kirkland, J.J.; *J. Chromatogr.*, 352 (1986) 275.
85. Slayter, E.M.; *Grolier Multimedia Encyclopedia*, V. 7.02, 1995.
86. Leitz, E.; *Microscope* 34 (1986) 284. Quoted from Chapter XV, "Cautions in Viewing Objects", of H. Baker's *Of Microscopes and the Discoveries Made Thereby*; London, 1785.
87. *Axiomat Multimodule Microscope*, Instruction Manual, Leitz, Stuttgart, 1986.
88. Behrisch, R., Ed.; *Sputtering by Particle Bombardment*, Springer Series Topics in Applied Physics, 47, Springer, Berlin, 1981.
89. *Nanoscope III Multimode Scanning Probe Microscope Instruction Manual*, Version 1.5, Digital Instruments, Santa Barbara, CA, 1993.
90. Bottomley, L.A., Coury, J.E., First, P.N.; *Anal. Chem.* 68 (1996) 185R.
91. Frommer, J.; *Angew. Chem. Int. Ed. Engl.* 31 (1992) 1298.
92. Mate, M.; IBM Almaden Research Center, San Jose, CA, personal communication, 1996. Fundamental tunneling mechanisms on thin film surfaces are used to explain storage disk lubrication failures.
93. Griffith, J.E., Marchman, H.M., Miller, G.L., Hopkins, L.C.; *J. Vac. Sci. Technol. B*, 13 (1995) 1100.
94. Sarid, D., Elings, V.; *J. Vac. Sci. Technol. B*, 9 (1991) 431.
95. Binnig, G., Rohrer, H., Gerber, C., Weibel, E.; *Phys. Rev. Lett.*, 49 (1982) 57.
96. Tersoff, J., Hamann, D.R.; *Phys. Rev. B*, 31 (1985) 805.
97. Atkins, P.W., *Molecular Quantum Mechanics*, Oxford University Press, Oxford, 1983.
98. Binnig, G., Rohrer, H.; *IBM J. Res. Develop.*, 30 (1986) 355.
99. Eigler, D.M., Schweizer, E.K.; *Nature* 344 (1990) 524.
100. Zeppenfeld, P., Lutz, C.P., Eigler, D.M.; *Ultramicroscopy*, 42-44 (1992) 128.
101. Tsukada, M., Kobayashi, K., Nobuyuki, I., Kageshima, H.; *Surf. Sci. Rep.*, 8 (1991) 265.

102. Feenstra, R., Stroscio, J., Fein, A.; *Surf. Sci.*, 181 (1987) 295.
103. Hamers, R.J., Tromp, R.M., Demuth, J.E.; *Phys. Rev. Lett.*, 56 (1986) 1972.
104. Inaba, M., Siroma, Z., Funabiki, A., Ogumi, Z., Abe, T., Mizutani, Y., Asano, M.; *Langmuir*, 12 (1996) 1535.
105. Cornell University UHV/STM Laboratory; XSTM of Quantum Dots, <http://www.hgc.cornell.edu/uhvstm/cleomap/index.html>, 1999.
106. Quantum Corral: ref. in Eigler, D.M., Schweizer, E.K.; *Nature* 344 (1990) 524.
107. Takano, H., Kenseth, J.R., Wong, S.-S., O'Brien, J.C., Porter, M.D.; *Chem. Rev.*, 99 (1999) 2845.
108. Mate, M.; *IBM J. Res. Develop.*, 39 (1995) 617.
109. Martin, Y., Williams, C.C., Wickramasinghe, H.K.; *J. Appl. Phys.*, 61 (1987) 4723.
110. Frisbie, C.D., Rozsnyai, L.F., Noy, A., Wrighton, M.S., Lieber, C.M.; *Science*, 265 (1994) 2072.
111. Lee, G.U., Chrisey, L.A., Colton, R.J.; *Science*, 266 (1994) 771.
112. Ohnesorge, F., Binnig, G.; *Science*, 260 (1993) 1451.
113. Schmitz, I., Schreiner, M., Friedbacher, G., Grasserbauer, M.; *Anal. Chem.*, 69 (1997) 1012.
114. AFM Imaging Modes Technical Report, Topometrix: Santa Clara, CA, 1993.
115. Rutland, M.W., Senden, T.J.; *Langmuir*, 9 (1993) 412.
116. Digital Instruments; Probing Nano-Scale Forces with the AFM, <http://www.di.com/AppNotes/ForcCurv/FCMain.html>, 1999.
117. Yang, A.C.M.; *Mater. Chem. Phys.*, 41 (1995) 295.
118. Radmacher, M.; *Langmuir*, 10 (1994) 3809.
119. Juai, R., Bhushan, B.; *J. Mater. Res.*, 8 (1993) 3019.
120. Fujihira, M., Daisuke, A., Okabe, Y., Takano, H., Hokari, H., Frommer, J., Nagatani, Y., Fumiki, S.; *Chem. Lett.*, 499 (1999).
121. Bhushan, B., Ed.; *Handbook of Micro/Nano Tribology*, CRC Press, Boca Raton, 1995.
122. Ito, T., Namba, M., Buhlmann, P., Umezawa, Y.; *Langmuir*, 13 (1997) 4323.

123. Wenzler, L.A., Moyes, G.L., Olson, L.G., Harris, J.M., Beebe, Jr., P.T.; *Anal. Chem.* 69 (1997) 2855.
124. van der Vegte, E.W., Hadziioannou, G.; *Langmuir* 13 (1997) 4357.
125. Burnham, N.A., Colton, R.J.; *J. Vac. Sci. Technol. A*, 7 (1989) 2906.
126. Probes on wafers purchased from Park Scientific (Santa Clara) and Nanoprobe (from Digital Instruments, Santa Barbara); 1997.
127. *Nanoscope Command Reference Manual*, V. 4.10, Digital Instruments: Santa Barbara, CA, 1995.
128. Wen, Y.H., Wu, S.S., Wu, H.L.; *J. Liq. Chromatogr.*, 18 (1995) 3329.
129. Zhen, Z.J., Wang, Z.G., Dong, Y.H., Hu, C.H., Yang, S.D.; *Clin. Chem.*, 41 (1995) S35.
130. Da, S.L., Yue, W.G., Wen, Y.F., Da, H.L., Wang, Z.H.; *Anal. Chim. Acta*, 299 (1994) 239.
131. Nobuhara, K., Kato, M., Nakamura, M., Takami, M., Kaneko, S.; *J. Chromatogr. A*, 704 (1995) 45.
132. Anderson, D.J.; *Anal. Chem.*, 67 (1995) R475.
133. Magonov, S.N., Heaton, M.G.; *Am. Lab.*, April (1996) 59.
134. Zhong, Q., Inniss, D., Kjoller, K., Elings, V.B.; *Surf. Sci. Lett.*, 290 (1993) L688.
135. Kaupp, S., Watzig, H.; *J. Chromatogr. A*, 781 (1997) 55.
136. Cifuentes, A., Diez-Masa, J.C., Friz, J., Anselmetti, D., Bruno, A.E.; *Anal. Chem.*, 70 (1998) 3458.
137. Ando, A., Miki, K., Matsumoto, K., Shimizu, T., Morita, Y., Tokumoto, H.; *Jpn. J. Appl. Phys.*, 35 (1996) 1064.
138. *RQS-RMS Microroughness Standard: Background Theory and Application Notes*, V. 1.5, VLSI Standards (1996).
139. Frommer, J.; IBM Almaden Research Center, San Jose, CA, personal communication, 1997.
140. Barrat, A., Silberzan, P., Bourdieu, L., Chatenay, D.; *Europhys. Lett.*, 20 (1992) 633.
141. Peach, S., Polak, R.D., Franck, C.; *Langmuir*, 12 (1996) 6053.

142. Fujii, M., Sugisawa, S., Fukada, K., Kato, T., Sirakawa, T., Seimiya, T.; *Langmuir*, 10 (1994) 984.
143. Fujihira, M., Morita, Y.; *J. Vac. Sci. Technol. B*, 12 (1994) 1609.
144. Ando, A., Miki, K., Matsumoto, K., Shimizu, T., Morita, Y., Tokumoto, H.; *Jpn. J. Appl. Phys.*, 34 (1995) 715.
145. Simpson, G.J., Sedin, D.L., Rowlen, K.L.; *Langmuir*, 15 (1999) 1429.
146. Correlation plots of all quantitative data measured in this report were made with reference to scan size. Results concur with reference 145: Simpson, G.J., Sedin, D.L., Rowlen, K.L.; *Langmuir*, 15 (1999) 1429.
147. Ducker, W.A., Senden, T.J., Pashley, R.M.; *Nature (London)* 353 (1991) 239.
148. Griffith, J.E., Grigg, D.A.; *J. Appl. Phys.*, 74 (1993) R83.
149. Cook, D.; Digital Instruments, Santa Barbara, personal communication, 1999.
150. Jaccodine, R.J.; *J. Appl. Phys.*, 33 (1962) 2643.
151. Flidr, J., Huang, Y-C., Newton, T.A., Hines, M.A.; *J. Chem. Phys.*, 13 (1998) 5542.
152. Johnson, K.L., Kendall, K., Roberts, A.D.; *Proc. R. Soc. London A*, 324 (1971) 301, in references 72 and 80.
153. Mate, M.; *J. Chem. Phys.*, 94 (1991) 8420.
154. Cataby, H., Sawada, H., Jinno, K., Pesek, J.J., Matyska, M.T.; *J. Cap. Electrophor.*, 5 (1998) 89.

Figure 1. Optical images of a fused silica OTCEC tube sheathed with polyimide: a) transverse section (x200); b) longitudinal section (x100).

Figure 2. Instrument configured for HPCE or OTCEC separations. After a sample is injected from vial (1), the capillary is moved to the source (mobile phase) vial (2) for the remainder of the experiment.

Figure 3. Transverse section SEM image (x20,000) of a 20 μm i.d. etched capillary tube. Plates, observed optically in reference 29, and columnar remnants of aggressive etching block the OTCEC tube.

SIGNAL BEAM RAY PATHS IN THE SEM, AFM, AND REFLECTED LIGHT MICROSCOPIES

Figure 4. The signal beam (ray) path in a SEM (a) is focused electromagnetically, and a reflection light microscope (c) is focused with optical condensor and objective lenses. Inversion of (c) gives a ray path analogous to the SEM. The laser beam reflected by an AFM cantilever (b) is optically reflected to its detector.

Figure 5. Phase-contrasted (a) and plane-polarized, phase-reversed (b) optical images (x1200) of an alkyl-derivatized capillary tube illustrate feature resolution in light microscopy. Boundaries of the inner bore in this longitudinal section are roughly one-third and two-thirds below the top of each photograph. Arrows indicate a like feature for image orientation. Image (a) shows authentic (dark-to-light, low-to-high) feature orientation.

Figure 6. Events responsible for a secondary electron image formed in SEM, occurring at and below a sample surface in response to a 10 - 50 kV electron beam.

Figure 7. Instrumental features of a field emission scanning electron microscope.

AFM TIP-SAMPLE FORCES OF INTERACTION

NATURE of INTERACTION	FORCE N	TIP-SAMPLE SEPARATION	FORCE CURVE REGION
Fluid Film (contaminant layer)	10^{-5}	$\approx 10 \mu\text{m}$	Attractive & Repulsive
Electrostatic	10^{-6}	$\approx 0 - 1 \mu\text{m}$	Attractive & Repulsive
	10^{-7}		
Capillary (fluid surface tension)	10^{-8}	$\approx 10 - 200 \text{ nm}$	Attractive
	10^{-9}		
van der Waal's	10^{-10}	$\approx 1 \text{ \AA}$	Attractive
Coulombic	10^{-10}	sub $\text{\AA}$	Repulsive
Contact		Zero	Repulsive

Figure 8. Tip-sample forces of interaction as an AFM tip approaches a sample surface.

NOTE TO USERS

Page(s) not included in the original manuscript are unavailable from the author or university. The manuscript was microfilmed as received.

95

This reproduction is the best copy available.

UMI

Figure 10. Optical image ($\approx \times 50$) of a silicon nitride probe engaged with the surface of a $50\ \mu\text{m}$ i.d. capillary bore in contact mode AFM. The small cantilever is not engaged.

Figure 11. SEM image (x10,000) of a silicon nitride contact mode AFM probe tip (beneath a cantilever similar to that in Figure 10) after use on hard, i.e., quartz and mica surfaces.

Figure 12. SEM images of: (a) an unused silicon AFM intermittent mode substrate-mounted cantilever with tip just visible near the right cantilever end (x500); (b) the silicon AFM tip on the same cantilever (x5000). Crater visible in (b) is apparently a defect.

Figure 13. SEM image ($\times 10,000$) of a silicon intermittent mode AFM tip damaged by excessive force applied by the z-piezo scanner. The remnant pyramid in the center was still capable of imaging smoother (e.g., native silica or alkylated) surfaces.

Figure 14. Idealized AFM force curve illustrates the cantilever deflection behavior with respect to z-piezo position during a full approach-retraction cycle force measurement. Arbitrary units of voltage or distance apply for the x- and y-axes.

Figure 15. Instrumental features of an atomic force microscope. The scanner approaches the probe in this configuration. A laser source is mounted above the probe in the optical head, and a mirror and photodiode detector are located within the optical head.

Figure 16. Gas chromatograph modified for reagent flow, capillary heating, and atmospheric control during OTCEC capillary tube derivatization.

Figure 17. SEM (x5000) view into an etched 50 μm i.d. OTCEC capillary tube. Surface artifacts are fused silica slivers from the sample preparation, discernible as separate phases in AFM amplitude deflection (constant height) mode images.

Figure 18. Force curve generated during an AFM adhesion force measurement of a native capillary tube surface. A small tip snap is visible just above the set point line. Abscissa scale = $0.07 \mu\text{m}/\text{div}$, ordinate = $0.16 \mu\text{m}/\text{div}$. Force measurement was taken with a set point voltage of -0.75 V , z-scan rate of 1.25 Hz , and z-scan size of $1.40 \mu\text{m}$.

Figure 19. AFM cross section analysis of a native fused silica surface: (a) line indicates the region in the image x,y-plane where a z-plane section (b) is rendered for offline analysis. Arrows in both $5\ \mu\text{m} \times 5\ \mu\text{m}$ scan images coincide, which allow vertical and horizontal measurements ($49.75\ \text{nm}$ and $2.5\ \mu\text{m}$ in this image) of regions of interest.

Figure 20. AFM $3\ \mu\text{m} \times 3\ \mu\text{m}$ ($z = 200\ \text{nm}$) intermittent mode images of octadecyl-derivatized surfaces using the hydrosilation technique on: (a) a flat silica (oxidized Si) wafer and (b) inside a $50\ \mu\text{m}$ i.d. OTCEC tube. Conventional z-dimension gray scale of dark (low) to light (high) is optimized for resolution in all AFM images presented here.

Figure 21. AFM ($z = 30$ nm, 300 nm, 1000 nm, and 750 nm, respectively) and SEM ($\times 20,000$) $3\ \mu\text{m} \times 3\ \mu\text{m}$ images at each of four stages of OTCEC derivatization.

Figure 22. AFM ($z = 5$ nm, 500 nm, 1000 nm, and 350 nm, respectively) $3\ \mu\text{m} \times 3\ \mu\text{m}$ images at each of four stages of OTCEC derivatization rendered in 3-D.

Figure 23. AFM $1\ \mu\text{m} \times 1\ \mu\text{m}$ (left) and $5\ \mu\text{m} \times 5\ \mu\text{m}$ (right) images of the four stages of capillary modification rendered in 3-D. AFM z-heights (100 nm, left, and 200 nm, right) have been scaled equally to emphasize topographic variations between surfaces.

Figure 24. AFM intermittent mode images of native silica OTCEC tube surfaces.
(a) $5\ \mu\text{m} \times 5\ \mu\text{m}$, $z = 6.5\ \text{nm}$. (b) $1.5\ \mu\text{m} \times 1.5\ \mu\text{m}$, $z = 8.1\ \text{nm}$. Holes seen in image (b) are believed to be manufacturing defects.

Figure 25. AFM intermittent mode images of an etched silica OTCEC tube surface. (a) $5\ \mu\text{m} \times 5\ \mu\text{m}$, $z = 295\ \text{nm}$. (b) $3\ \mu\text{m} \times 3\ \mu\text{m}$, $z = 270\ \text{nm}$ scan of upper left in (a).

Figure 26. AFM contact (a) and intermittent (b) mode images of etched silica OTCEC tube surfaces demonstrate reproducibility between different capillary surfaces and AFM modes. (a) $1\ \mu\text{m} \times 1\ \mu\text{m}$, $z = 33.9\ \text{nm}$. (b) $1\ \mu\text{m} \times 1\ \mu\text{m}$, $z = 38.9\ \text{nm}$.

Figure 27. AFM intermittent mode phase image of the etched surface in Figure 25a.
Area in lower right has a tip artifact, probably due to excessive drive amplitude.

Figure 28. (a) AFM ($z = 200$ nm) and (b) SEM ($\times 120,000$) $1\ \mu\text{m} \times 1\ \mu\text{m}$ images of holes in a mid-sized range (300 nm) found on hydride-modified OTCEC tube surfaces. The general morphology is seen to be duplicated, as a depression in (a) is probed deeper. Repeating plate units are seen left of below center in (b).

Figure 29. High resolution SEM image (x 50,000) of radial plate extensions broken after extensive etching in a narrow bore (20 μm i.d.) tube provides further evidence of geometric duplication in the derivatization of capillary surfaces.

Figure 30. SEM image (x1000) of an oblique section of an etched, hydrided (50 μm i.d.) OTCEC tube. Holes and plates are just visible inside the tube.

Figure 31. (a) Intermittent mode AFM image ($5\ \mu\text{m} \times 2.5\ \mu\text{m}$, $z = 550\ \text{nm}$) of a hydrided OTCEC capillary inner bore surface. Arrows indicate duplicate plate structures. (b) AFM phase image ($5\ \mu\text{m} \times 5\ \mu\text{m}$, $z = 750\ \text{nm}$) of growth plates oriented normal, parallel, and oblique to a hydrided capillary bore surface. Thin structures may be plate edges.

Figure 32. AFM intermittent mode images of etched, octadecyl-derivatized silica OTCEC tube surfaces. (a) $3\ \mu\text{m} \times 3\ \mu\text{m}$, $z = 177\ \text{nm}$. (b) $3\ \mu\text{m} \times 3\ \mu\text{m}$, $z = 125\ \text{nm}$.

Figure 33. (a) AFM ($z = 200$ nm) and (b) SEM ($\times 120,000$) $1\ \mu\text{m} \times 1\ \mu\text{m}$ images of medium range, 300 to 400 nm (a) and largest range, 500 to 800 nm (b) pits found on octadecyl-derivatized silica OTCEC tube surfaces.

Figure 34. Distribution of rms roughnesses of the four stages of capillary modification.

Figure 35. Distribution of surface areas of the four stages of capillary modification.

Figure 36. Distribution of surface-tip forces of the four stages of capillary modification. Hydrided (I) and (II) represent distinct force populations found on these surfaces.

Figure 37. (a) Force curve measured in a region of an etched OTCEC tube (near center of the box) in (b) on a surface possibly passivated by silica corrosion. Image parameters ($1\ \mu\text{m} \times 1\ \mu\text{m}$ scan, $z = 50\ \text{nm}$) and probe are the same used on the surface in Figure 38.

Figure 38. (a) Force curve measured in a region of an etched OTCEC tube (near center of the box) in (b) on a possibly activated silica surface. Image parameters ($1\ \mu\text{m} \times 1\ \mu\text{m}$ scan, $z = 50\ \text{nm}$) and probe are the same used on the surface in Figure 37.

	RMS Roughness nm (a)	Surface Area % Difference	Tip-Sample Force nN (b)
(c) NATIVE	0.8 (0.3)	0.2 (0.2)	5.0 (1.0)
ETCHED	15 (11)	3.9 (4.5)	28 (16)
HYDRIDED	33 (23)	17 (12)	I: 5.0 (2.0) II: 42 (11)
ALKYLATED	16 (9)	7.7 (6.8)	5.0 (1.0)

(a) Standard Deviation (σ) in parentheses.

(b) Hydrided I and II are distinct force populations found on these surfaces (see Figure 36).

(c) Sample count may be viewed in the histogram frequency plots for each measurement, Figures 34 - 36.

Table I. Summary of mean values and standard deviations of the RMS roughnesses (Rq), surface area % differences, and surface-tip adhesive forces for the four stages of capillary modification, as determined by AFM topographic image height measurements.