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Raman spectroscopy for quantification of polydimethylsiloxane concentration in turbid samples

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Abstract

This paper presents a preliminary application of Raman spectroscopy in conjunction with the chemometric method of Partial Least Squares to predict silicone concentrations in homogenous turbid samples. The chemometric technique is applied to Raman spectra to develop an empirical, linear model relating sample spectra to polydimethysiloxane (silicone) concentration. This is done using a training set of samples having optical properties and known concentrations representative of those unknown samples to be predicted. Partial Least Squares, performed via cross-validation, was able to predict silicone concentrations in good agreement with true values. The detection limit obtained for this preliminary investigation is on par with that of magnetic resonance spectroscopy. The data acquisition time for this Raman based method is 200 seconds, which compares favorably with the 17 hour acquisition required for magnetic resonance spectroscopy to obtain a similar sensitivity. The combination of Raman spectroscopy and chemometrics shows promise as a tool for quantification of silicone concentrations from turbid samples.

Key Words: Raman Spectroscopy, Optical Spectroscopy, Biomedical Diagnostics, Partial Least Squares, Polydimethylsiloxane (PDMS), Breast Implant Leakage, Silicone

1.Introduction

Silicone-gel implants for breast augmentation and reconstruction have been in use since 1962. In April of 1992, the Food and Drug Administration (FDA) restricted the use of silicone gel filled breast implants, citing lack of adequate information on health risks associated with such implants.[1,2] Local complications have long been known to occur, primarily consisting of capsular contracture, a hardening of the implant to palpation due to contracture of the fibrous capsule that normally forms around the implanted body.[3,4,5] Ruptures of implants can occur either intracapsularly, or with extracapsular extension and concomitant spread of the gel to the adjacent tissue.[6,7,8] While the long term effects of silicone leakage remain uncertain, published studies to date suggest a rupture rate between 5 and 51 percent.[2] The uncertainty in rupture rates is largely due to the lack of a reliable method for detection and quantification of silicone leakage. Virtually all implants have been shown to "bleed" silicone through their packaging into the local micro-environment .[9,10] This is supported by histologic findings of foreign-body granulomas in the capsular tissues or regional lymph nodes.[7,11] More recent observations using magnetic resonance spectroscopy (MRS) have suggested the presence of silicon compounds in the blood of some women with silicone breast implants, as well as evidence of silicon migrating to the liver.[12]

A recent article in the New England Journal of Medicine indicates that there may be a small long term risk associated with silicone implants. However, this study is epidemiological and by no means conclusive.[13] As a scientific adjunct to studies of this type, a means for non-invasive determination of the status of implants, and quantification of silicone levels in blood and tissue continues to be a goal of the medical community . [14-18]

Many technologies are currently under investigation for use as potential tools for detection of silicone, but for the most part these suffer from a host of practical difficulties. Although computed tomography (CT) scans produce high resolution images,[15] patients are exposed to ionizing radiation; therefore CT is not recommended for patients with augmentation mammoplasty.[10] Standard light microscopy histopathologic techniques have been shown to be ineffective because silicone is refractile, nonpolarizable, nonstainable and therefore difficult to

identify.[19] Ultrasonography has been investigated as a method of evaluation of implant integrity but is insensitive to detection of silicone "bleed-through".[20] Several laboratories are offering tests that claim to detect levels of antibodies to silicone that presumably indicate a leaking or ruptured implant. However, the very existence of such silicone antibodies has not yet been conclusively.[10] There are claims that extremely high antibody levels may indicate a leaking or ruptured implant. However the clinical significance of silicone antibodies and at what levels these antibodies are harmful remains unknown.[16,17] While magnetic resonance spectroscopy (MRS) techniques are being investigated to identify implant leaks, they generally suffer from lack of sensitivity in detecting silicone either in small amounts or when silicone is heterogeneously distributed in tissue.[12] In addition, MRS is expensive and time consuming. Finally, standard analytical chemistry techniques from such as Direct Current Plasma-Atomic Emission Spectroscopy (DCP-AES) can be used to detect elemental Si *in vitro* with a sensitivity of 2.0 μg/gm (tissue).[18] These methods however cannot differentiate between sources of silicone (PDMS) and elemental silicon, which is often present in non-implant individuals at measurable levels. In addition, these spectroscopic techniques are sample destructive, require extensive sample preparation and are not readily accomplished in a clinical setting.

There is not yet a reliable noninvasive technique for determining leakage outside of fibrous capsule. It is the goal of the present work to accomplish that task through an optical method. In addition a functional optical technique, but could potentially aid in the general understanding of the problems associated with *in vivo* optical diagnostics and tissue biochemistry.[10]

Optical spectroscopic methods have become the subject of considerable study as a tool for monitoring the state of tissue. The radiation that is used for these studies, which encompass Raman spectroscopy,[21] fluorescence spectroscopy,[22] Fourier transform infrared spectroscopy (FT-IR),[23,24] and reflectance spectroscopy,[25] is non-ionizing and is amenable to transmission via optical fiber. Spectral data can therefore be acquired using a contact or minimally invasive probe and the efficacy of these various spectroscopies can be studied at a number of organ sites without causing the patient undue risk or discomfort. Although many biologically important molecules can be probed using optical spectroscopy, only limited attempts have been made to interpret tissue Raman spectra in terms of tissue chemical composition.[13,26,27] Raman spectroscopy is particularly attractive because it is a proven tool for sensitive identification of molecular constituents in a mixture of unknowns for non-biomedical applications.[28]

We have employed the method of Partial Least Squares (PLS) to develop an empirical linear model of Raman scattering in turbid samples using a training set with known silicone concentrations and optical properties similar to those considered as unknowns.[29] Recently, efforts have been made to quantify chromophores in biological samples using PLS, where work has focused on the determination of blood glucose levels using FT-IR techniques. These preliminary studies have shown promise in the accurate determination of quantitative information from optical spectra both *in vitro* and *in vivo*.[23,24]

The accuracy of prediction for PLS depends on the composition of the training and validation sets as well as the spectral information included in the data.[31] One technique used to assess the accuracy of prediction and to select the optimum number of factors to retain in the model is known as the method of cross-validation.[24,30] This technique evaluates the ability of a PLS calibration model to predict the concentrations of unknown spectra as a function of the rank (number of factors or principal components) used in creating the calibration model. A detailed discussion of cross-validation can be found in the literature.[30]

An attractive facet of PLS is that it does not explicitly require *a priori* knowledge of the sample optical properties but does require spectra from a training set of samples with known concentrations and with chemical complexity similar to the unknown sample of interest.[24,31,32] Biological systems are generally complex mixtures for which acquisition of complete chemical

information is time consuming and complex, if not impossible. Consequently, the application of PLS methods to Raman spectra, presents great potential as a method for optical determination of tissue biochemistry.

Figure 1 illustrates the unique Raman "fingerprint" of PDMS. This is a typical Raman spectrum of pure PDMS (viscosity = 200 centistokes) illustrating the Si-O stretch and Si-C stretch modes that are easily detectable at 491 cm-1 and 713 cm-1, respectively. These observed Raman modes are highly accurate and can be used as specific markers for all silicone products exhibiting unaltered silicon-oxygen and carbon-silicon bands.[33] This region of the Raman spectrum is particularly attractive due to the lack of interfering Raman signal from endogenous tissue constituents.[1,26,33]

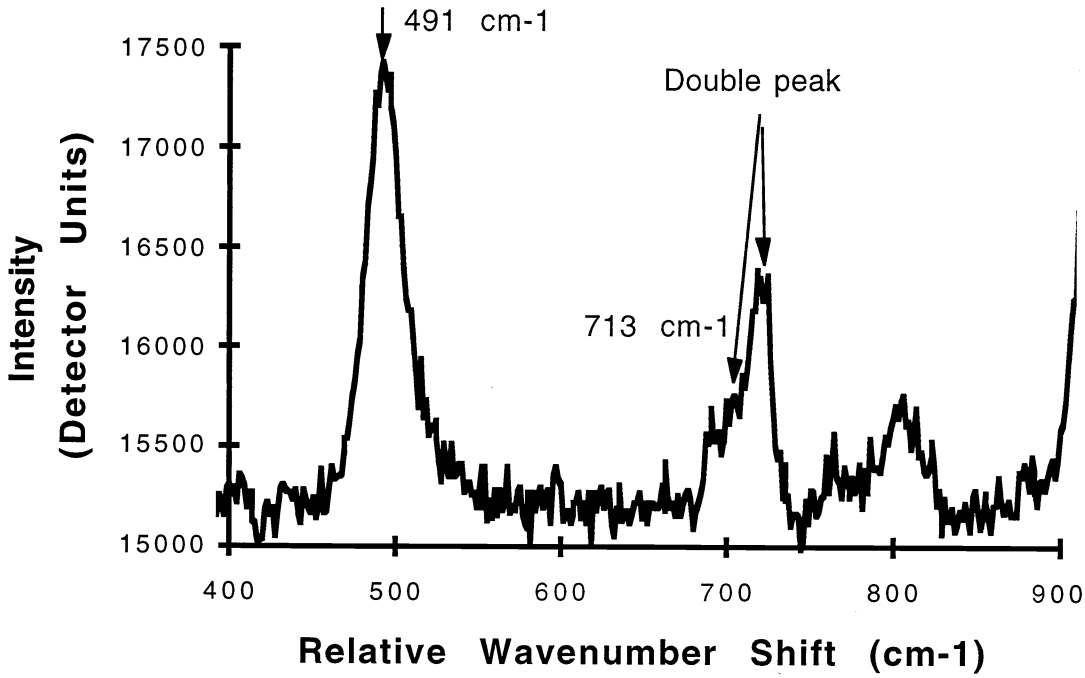

Figure 1: PDMS spectrum. Molecular structure: Si-O-Si- backbone with Methyl groups (-CH_3) bonded to the Si atoms Note: viscosity = function of cross-linking between carbons of Methyl groups. Key Raman features: Si-O stretch (491 cm^{-1}), Si-C stretch (713 cm^{-1}) location of these features is invariant with concentration (50 to 500 centistokes). Uniqueness: These observed Raman modes are highly accurate and serve as specific markers for all silicone products exhibiting unaltered silicon-oxygen and carbon-silicon bands.

2.Materials & Methods

To our knowledge, there has not yet been a study that explicitly examines the ability of PLS to predict chromophore concentrations from Raman spectra of turbid media such as human tissue. For PLS to accurately predict concentrations of chromophores in tissue, it must be shown that the technique can be employed as an accurate predictor in samples that absorb and scatter light. In order to examine the predictive ability of PLS using Raman spectroscopy in a tissue-like environment, we performed our study on a series of 40 turbid phantoms with optical properties similar to those of tissue.[22,29]

Polydimethylsiloxane (PDMS) was purchased from Sigma Chemical and used without further purification. A preliminary examination of the Raman spectra from pure PDMS gels with viscosity ranging from 20 centistokes (MW = 2000), to 500 centistokes (MW = 17,250) confirmed that the transitions of interest for the purposes of this investigation can in all cases be found at 491 cm^{-1} and 713 cm^{-1}. It was necessary to include an emulsifying agent in our phantoms so that PDMS would remain diffusely distributed in these mixtures. Sodium Dodecyl Sulfate (SDS, Sigma Chemical), a common non-ionic surfactant, was first checked for potentially interfering Raman scattering in the wavenumber region of interest and was subsequently mixed with silicone and phosphate-buffered saline (PBS). The introduction of this ingredient to the mix caused microglobularization of the silicone, resulting in a range of silicone microsphere diameters on the order of 20 to 100 μm, consistent with silicone particle sizes that have been observed *in vitro* investigations performed by other groups.[34,35]

Hemoglobin was obtained from blood drawn from the author. Serum was separated from whole blood and discarded according to laboratory standards for the handling of potentially biohazardous fluids. The remaining cells (primarily red cells) were placed in isotonic PBS (pH 7.4). This was used as a source of oxyhemoglobin for the remainder of the study. Using previous work with phantoms for quantitative fluorescence spectroscopy as a guideline, samples were mixed so that the final concentration of hemoglobin used in each turbid sample was between 0 and 2 % by volume (0 to 94.6 mM) over the set of 40 samples.[22] A spreadsheet using random number generators and statistical correlation functions was employed in order to insure that correlation between concentrations of the sample ingredients were minimized. This ensured that the model resulting from the PLS routine would not incorporate artifacts related to simple correlation between sample ingredients or the order in which sample spectra undergo the PLS decomposition . After placing PDMS, hemoglobin and surfactant in a non-silicone coated test tube, PBS was added to bring the final volume of each sample up to 10 ml. Spectra of the phantoms were acquired using the Raman system described in the next section.

The Raman spectrometer used in these studies is depicted in Fig. 2. The optical path, optical fiber collection bundle, cuvettes and test tubes used in this investigation were all tested for endogenous silicone contamination and interference in the Raman regime of interest to minimize the possibility of corrupting our experiment. Light from a 632.8 nm helium neon (HeNe) laser (Spectra-Physics 127) irradiated a plastic cuvette containing approximately 3 ml of sample material. A line pass filter was used to prevent HeNe plasma lines from reaching the sample. Scattered light was collected from the sample using an optical fiber bundle placed in close proximity to the illuminated sample spot. Rayleigh scattered light was prevented from reaching the detector using a line filter (Kaiser Super Notch, 6 OD, 632.8 nm). Light entering the spectrograph (SPEX 270 f/4, slit size = 50μm) was dispersed across the face of a CCD detector using a holographic grating (blaze angle = 630 nm, 1200 grooves/mm, RLD: 3.1 nm/mm). The detector was an intensified red/blue enhanced CCD camera (Princeton Instruments ICCD-576G/RBT , 576 x 384 array, pixel size = 22 x 22 μm, active area = 12.7 x 8.4 mm, peak quantum efficiency = 40%). The CCD was thermoelectrically cooled to an operating temperature of -40 C.

Calibration and data acquisition were performed using Kestrel Spec software (Rhea Corp.). The 632.8 nm neon line was used with the single point calibration routine provided in the

software. Reproducibility of the calibration was verified using the HeNe plasma lines which could be permitted to reach the detector by removing the line pass filter. The integration time for each acquired spectrum was 1 second with a total of 200 accumulated spectra per sample. In order to simulate a device that would not compromise the integrity of whole blood cells, data collection parameters were loosely based on parameters used by various other groups involved in Raman studies of tissues.[1,26] Real time compensation for fluctuations in laser power was not possible in this case because a reliable cw detector was not available. However, we were able to account for the slow decrease in laser power over the course of the experiment. To do this, a "standard" Raman spectrum was acquired, using parameters identical to those used for the turbid samples, from a cuvette containing pure 200 centistokes PDMS every 15 minutes. This was used to normalize the turbid spectra taken in each 15 minute segment. Data were then exported to Microsoft Excel and combined to form matrices. MATLAB (the Math Works, Natick MA) was subsequently used to execute PLS and cross-validation routines.

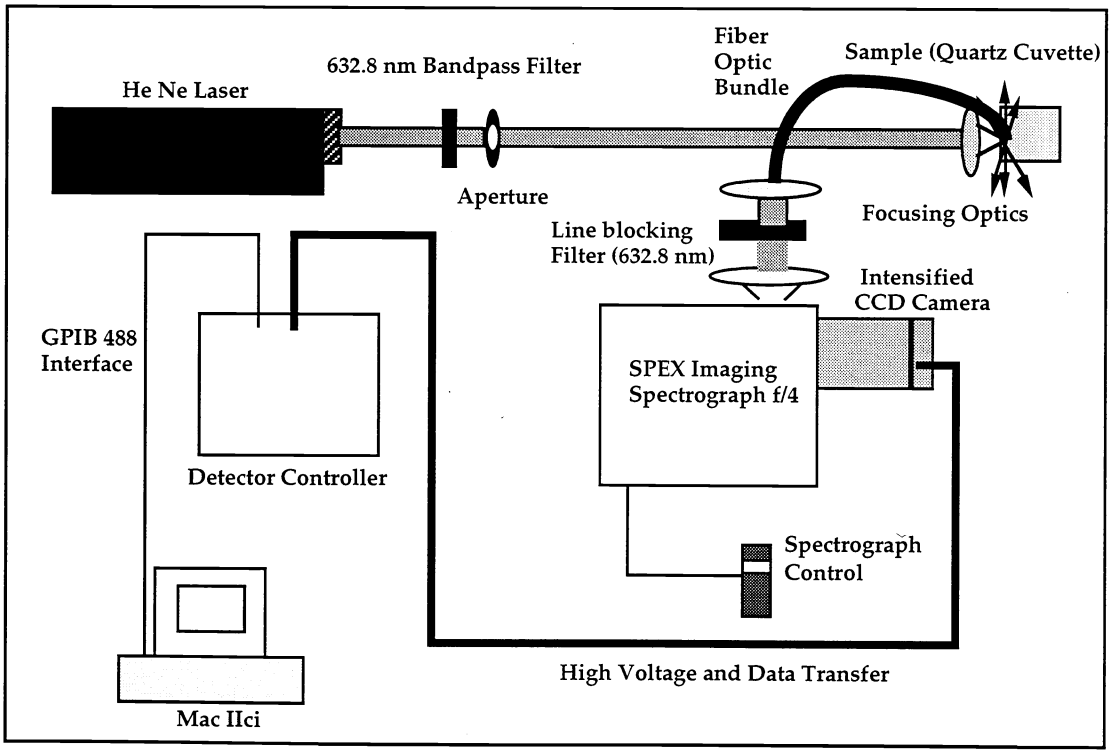

Figure 2: Experimental Setup. The Raman Spectrometer constructed for our preliminary investigations. The source is a 35 mW HeNe laser. Raman spectra were acquired using a cooled, intensified CCD.

Data from the turbid samples were analyzed using a Partial Least Squares algorithm from the MATLAB Chemometrics Toolbox. The most accurate prediction model for the training data was chosen using a cross-validation algorithm which would iteratively construct a model for N-1 spectra and treat the remaining spectrum as the "unknown". The matrix of the N-1 spectra was then decomposed into principal components, or factors, using singular value decomposition.[36] Models were built using a successively increasing number of these factors. On the first iteration, the model consists of only the factor that accounts for most of the variance in the data (indicated by the magnitude of the corresponding Eigenvalue). The model for each factor level was applied to the "unknown" spectrum to predict the concentration of the component of interest and the prediction

error was recorded in a matrix for future reference. The next most important factor (indicated by the magnitude of the corresponding eigenvalue) was subsequently included in the model and the prediction process repeated until a model consisting of all factors resulting from the decomposition of the N-1 training set was employed. The "unknown" was then returned to the training data and a different spectrum was selected as the "unknown." This entire process was repeated until all samples had played the role of "unknown" once. The prediction error for each factor level was summed across the sample set and plotted. The model for the entire set was then constructed using the factor level for which the residuals across the sample set were minimized. Models consisting of the "optimum" number of factors+1 and the "optimum" number of factors-1 were also constructed. These models were also applied to the data and the predictions compared to the results obtained using the "optimum" number of factors.

3.Results

Figure 3 illustrates the predicted PDMS concentrations obtained by applying PLS to the data acquired for the turbid phantoms. The sum of the residual errors incurred via cross-validation were determined as a function of the number of factors. These results indicated that a model based on 4 factors yields the first minimum in residual prediction error across the sample set. This is a reasonable outcome considering that there are 4 constituents in each mixture. The arrow in Fig. 3 indicates recent *in vitro* results obtained using MRS on a blood sample taken from a patient with silicone filled implants.[12] This corresponds to a PDMS concentration of about 5 mg/gram.

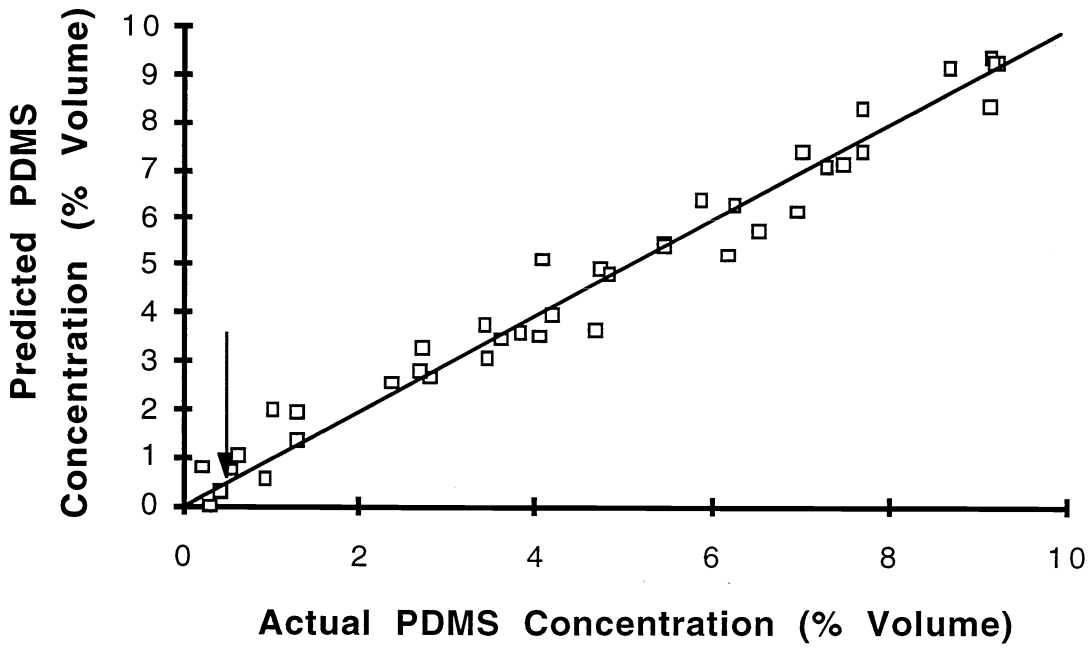

Figure 3: **Results.** Prediction of PDMS concentration in turbid samples. The diagonal line has a slope =1. In an "ideal" investigation, all predictions would lie along this line. The arrow indicates recent *in vitro* results obtained using MRS on a blood sample taken from a patient with silicone filled implants .[12] This corresponds to a PDMS concentration of 5 mg/gram of tissue. Our preliminary results based on Raman spectroscopy and PLS compare favorably with this result.

4.Discussion

The results of this work indicate that Raman spectroscopy has potential to be useful for the discrete detection of low-concentration foreign compounds in a turbid optical environment. We were able to obtain good agreement between measured and predicted silicone concentrations in optically complex samples over a wide range of optical properties. Considerable work remains to be done to explore the detection limits of this technique. One obvious improvement can be made by introducing an additional detector to compensate for fluctuations in laser power at the same time data from turbid samples is acquired In the future we intend to make the following improvements in our to gain a greater understanding of the practical limits of this technique:

In the intensity regime appropriate to medical diagnostics, the Raman signal will scale linearly with increasing laser power. Consequently, considerable signal enhancement can be realized by use of a diode laser (~350 mW @ ~800 nm) compared to the current 632 nm HeNe laser (~15 mW).

We estimate the collection efficiency for Raman-scattered radiation for these preliminary experiments to be only a few percent. This leaves considerable room for improvement through modifications to the optical geometry. Toward this end, there have been recent reports of progress in assembling an optical-fiber-based Raman system for biomedical applications. Specifically, a spectroscopy group at MIT has recently demonstrated a 7-fold increase in collection efficiency of Raman scattered radiation from a tissue surface, using a parabolic reflecting element coupled to an optical fiber.[37] In addition, a group at the University of Texas has had success in obtaining *in-vivo* measurements using an optical probe designed to improve optical throughput and reduce interfering Raman signal from the fiber probe itself.[26]

Finally, application of the Partial Least Squares (PLS) method for spectral quantitative analysis is a very active field that is continuously being refined and improved. To date we have applied PLS to the problem of silicone bioassay in a cursory manner. A review of the literature indicates that refinements in both data pre-processing and post-processing can be used to enhance the predictive ability of the method.[38] These enhancements will be augmented in a synergistic manner by the better Raman spectra (in terms of signal-to-noise ratio) we hope to obtain through the aforementioned modifications.

Should these attempts at assay enhancement prove beneficial, a successful diagnostic technique could readily be incorporated into one of the various instruments being developed for optical imaging of the breast. Broader public health benefits include possible low-cost sensitive assays of breast disease, that can be readily integrated into an imaging system. Techniques developed could conceivably be extended to spectroscopic investigations in other organ sites and ultimately may provide a means by which metabolic changes of *in vivo* tissue can be monitored.

5.Acknowledgments

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6.References

1 C.J. Franck, R.L McCreery, D.C.B. Redd, T.S. Gansler, "Detection of Silicone in Lymph Node Biopsy Specimens by Near-Infrared Spectroscopy", Applied Spectroscopy 47, No. 4, 387 (1993).

2 D.A. Kessler, "The basis of the FDA's decision on breast implants", New England Journal of Medicine, 326:1713 (1992).

3 L. Gylbert, O. Asplund, G. Jurell, "Capsular contracture after breast reconstruction with silicone-gel and saline-filled implants: A 6-year follow-up", Plast Reconstr Surg 85, 373(1990).

4 B. Brandt, V. Breiting, L. Christensen, M. Nielsen, J.L. Thomsen, "Five years experience of breast augmentation using silicone gel prostheses with emphasis on capsule shrinkage", Scand J. Plast Reconstr Surg18, 311 (1984).

5 O. Asplund, "Capsular contracture in silicone gel and saline-filled breast implants after reconstruction", Plast Reconstr Surg 73, 270 (1984).

6 R.J. Hausner, F.J. Schoen, M.A. Mendez-Fernandez, W.S. Henly, R.C. Geis, "Migration of silicone gel to axillary lymph nodes after prosthetic mammoplasty", Arch Pathol Lab Med 105, 371 (1981).

7 L.D. Truong, J. Cartwright Jr, M.D. Goodman, D. Woznicki, "Silicone lymphadenopathy associated with augmentation mammoplasty--Morphologic features of nine cases", Am J Surg Pathol 12, 484 (1988).

8 L. Garrido, B. Pfleiderer, B.C. Jenkins, C.A. Hulka, D.B. Kopans, "Migration and chemical modification of silicone in women with breast prostheses", Magn Reson Med 31, 328 (1994).

9 D.E. Barker., M.I. Retsky, S. Schultz, "Bleeding of silicone from bag-gel breast implants, and its clinical relation to fibrous capsule reaction", Plast Reconstr Surg; 61, 836 (1978).

10 S.S. Teuber, S.H. Yoshida, M.E. Gershwin, "Immunopathologic effects of silicone breast implants", The Western Journal of Medicine 162 No. 5, 418 (1995).

11 A. Bridges, C. Conley, D. Burns, F. Vasey, " A clinical and immunological evaluation of women with silicone breast implants and symptoms of rheumatic diseases." Ann. Intern. Med 118, 929 (1993).

12 B. Pfleiderer, J.L. Ackerman, L. Garrido, "Migration and biodegradation of free silicone from silicone gel-filled implants after long-term implantation", Magn Reson Med 30, 534 (1993).

13 J. Sanchez-Guerrero, G.A. Colditz, E.W. Karlson, D.J. Hunter, F.E. Speizer, M.H. Liang, "Silicone Breast Implants and the Risk of Connective-Tissue Diseases and Symptoms", The New England Journal of Medicine 1666 (1995).

14 J.A. Centeno, F.B. Johnson, "Microscopic Identification of Silicone in Human Breast Tissues by Infrared Microspectroscopy and X-Ray Microanalysis", Applied Spectroscopy 47 No. 3, 341 (1993).

15 C.Y. Ahn, N.D. DeBruhl, D.P. Gorczyca, L.W. Basset, W.W. Shaw. "Silicone Implant rupture Diagnosis Using Computed Tomography: A Case Report and Experience with 22 Surgically Removed Implants", Ann Plast Surg. 33, 624 (1994).

16 M.J. Rowley, A.D. Cook, S.S. Teuber, "Antibodies to collagen: Comparative epitope mapping in women with silicone breast implants, systemic Jupus erythematosus, and rheumatoid arthritis", J Autoimmun 7:775 (1994).

17 N. Kossovsky, M. Zeidler, G. Chun, "Surface dependent antigens identified by high binding avidity of serum antibodies in a sub-population of patients with breast prostheses", J Appl Biomaterials 4, 281 (1993).

18 G.R.D. Evans, S. Slezak, M. Rieters, G.M. Bercowy, "Silicon Tissue Assays in Nonaugmented Cadaveric Patients: Is there a Baseline level?", Plastic and Reconstructive Surgery May 22, 1117(1994).

19 D.S. Raso, W.B. Greene, J.J. Vesely, M.C. Willingham, "Light Microscopy Techniques for the Demonstration of Silicone Gel." Arch Pathol Lab Med 118 No.10, 984 (1994).

20 R.A. Levine, T. Collins, "Definitive Diagnosis of Breast Implant Rupture by Ultrasonography", Plast. Reconstr Surg. 86 No. 4, 803 (1990).

21 R. Manoharan, J.J. Baraga, M.S. Feld, R.P. Rava, "Quantitative histochemical analysis of human artery using Raman spectroscopy", J. Photochem. Photobiol. B: Biol 16, 211 (1992).

22 A.J. Durkin, R. Richards-Kortum, "A Comparison of Methods to Determine Chromophore Concentrations from Fluorescence Spectra of Turbid Samples", Lasers in Surgery and Medicine, 19 No.1, 75 (1996).

23 M.R. Robinson, R.P. Eaton, D.M. Haaland, G.W. Koepp, E.V. Thomas, B.R. Stallard, P.L. Robinson, "Noninvasive Glucose Monitoring in Diabetic Patients: A Preliminary Evaluation", Clinical Chemistry 38, 1618 (1992).

24 D.M. Haaland, "Multivariate Calibration Methods Applied to Quantitative FT-IR Analyses", Practical Fourier Infrared Spectroscopy. J.R. Ferraro, K. Krishnan Eds. (New York: Academic Press,1990), pp. 395-468.

25 I.J. Biggio, J.R. Mourant, J.D. Boyer, "Non-invasive identification of bladder cancer with subsurface backscattered light", Advances in Laser and Light Spectroscopy to Diagnose Cancer and other Diseases, R.R. Alfano, A. Katzir, Eds. (Bellingham, Washington, SPIE Press, 1994) 2135.

26 A. Mahadevan-Jansen, R. Richards-Kortum.,"Raman Spectroscopy for the Detection of Cancers and Precancers," Journal of Biomedical Optics 1 No.1, 31 (1996).

27 J.P. Wicksted, R.J. Erckens, M. Motamedi, M., " Monitoring of Aqueous Humor Metabolites using Raman Spectroscopy", Advances in Laser and Light Spectroscopy to Diagnose Cancer and other Diseases, R.R. Alfano, A. Katzir, Eds. (Bellingham, Washington, SPIE Press, 1994) 2135 pp. 264-274.

28 J.B. Cooper, P.E. Flecher, T.M. Vess, W.T. Welch, "Remote Fiber Optic Raman Analysis of Xylene Isomers in Mock Petroleum Fuels Using a Low-Cost Dispersive

Instrument and Partial Least-Squares Regression Analysis", Applied Spectroscopy 49 No.5, 586 (1995).

29 A.J. Durkin, S. Jaikumar, R. Richards-Kortum, "Optically Dilute, Absorbing and Turbid Phantoms for Fluorescence Spectroscopy of Homogeneous and Inhomogeneous Samples", Applied Spectroscopy 47 No.2, 114 (1993).

30 E.R. Malinowski., Factor Analysis in Chemistry, (New York, John Wiley and Sons, Inc. 1991) pp. 169-172.

31 A.J. Durkin, C.M. Gardner, R. Richards-Kortum, "Comparison of Methods for Determining Fluorophore Concentrations from Turbid Samples", Conference on Lasers and Electro-optics (CLEO), May (1994).

32 E.V. Thomas, D.M. Haaland, " Comparison of Multivariate Calibration Methods for Quantitative Spectral Analysis." Analytical Chemistry 62, 1091 (1990).

33 J.A. Centeno, J.L Luke, V.F. Kalasinsky, F.G. Mullick, "Biophysical Characterization of Silicone Breast Implants by Laser Raman Microprobe and Infrared Microspectroscopy" Preprints of Papers Presented at the 208th ACS National Meeting 34 No. 2, 132 (1994).

34 J.A. Centeno, V.F. Kalasinsky, F.B. Johnson, T.N. Vihn, T.J. O'Leary, "Fourier Transform Infrared Microscopic Identification of Foreign Materials in Tissue Sections", Laboratory Investigation 66 No.1, 123 (1992).

35 J.A. Centeno, F.B. Johnson, V.F. Kalasinsky, F.G. Mullick, J.P Pestaner, "Microspectroscopic Evaluation of Foreign Inclusions in Human Pathology: the Laser Raman Microprobe Technique." , Poster presented at United States and Canadian Academy of Pathology, Annual Meeting, Toronto, Canada (1995).

36 D.M. Haaland, E.V. Thomas, "Partial Least Squares Methods for Spectral Analysis 1: Relation to Other Quantitative Calibration Methods and the Extraction of Quantitative Information", Analytical Chemistry 601193(1988).

37 A.J. Berger, Y. Wang, D.M. Sammeth, I. Itzkan, K. Kneipp, M.S. Feld, "Aqueous Dissolved Gas Measurements Using Near-Infrared Raman Spectroscopy," Applied Spectroscopy 49,1164 (1995).

38 G.W. Small, M.A. Arnold, L.A. Marquardt, "Strategies for Coupling Digital Filtering with Partial Least Squares Regression: Application to the Determination of Glucose in Plasma by Fourier Transform Near-Infrared Spectroscopy," Analytical Chemistry 65, 3279 (1993).