

## A. Cover Page

State the course, Chemistry 3211 or 3281, the experiment number and title, the author's name, the partners' names, and the date submitted.

## B. Abstract (*one paragraph*)

In one paragraph containing several complete sentences, briefly describe:

- what the goal(s) of the experiment were
- how you attained those goals
- why those goals were important
- sample result(s) of the experiment (numerical data) (NOTE:  
don't include all your results here)
- concluding statement

The objective of this experiment was to determine the concentration of a phosphate solution. To achieve this, an automated flow injection analysis system was used, with multiple components adjusted as needed to obtain the best-defined peaks in the spectra. The end result was that greater proficiency with the FIA apparatus was gained, and the concentration was found without great difficulty (143.63 ppm). This method is a very useful one, especially since it has the added benefit of a large degree of automation.

### **Abstract**

Detection of small concentrations of phosphate is useful for studies monitoring marine phosphate cycles and groundwater contamination by fertilizer. The phosphate concentration can be determined by absorbance at 600 nm during flow injection analysis (FIA) using sequential reactions with molybdate and ascorbic acid. FIA allows the unstable products sufficient time to form without sitting for long periods of time. It was found that a reaction coil length of 50 cm for the molybdate reaction and 100 cm length for the ascorbic acid reaction produced the sharpest elution band in a short amount of time. A flow rate study showed the highest sensitivity but a long run time at 0.50 ml/min, and reduced sensitivity but a short run time at 1.56 ml/min. The best sensitivity/run time trade-off (1.10 ml/min) was chosen for unknown determination. A non-linear calibration curve was calculated from the peak area of standard solutions with a relative error of 2.33%. The phosphate concentration of an unknown solution was determined to be  $61.5 \text{ ppm} \pm 5.05\%$ . The experiment was thus a success.

## In Situ Determination of Lead in Paint by Laser-Induced Breakdown Spectroscopy Using a Fiber-Optic Probe

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On-site determination of leaded paint in houses is important for minimizing the costs of renovations. A simple fiber-optic probe suitable for remote elemental analysis using laser-induced breakdown spectroscopy has been developed for this purpose and is used to determine the lead concentration in samples of dry paint. Optical fibers transport the laser pulse to the sample and transfer the emission signal to the spectrometer. The use of separate excitation and collection fibers allows coupling of the probe to a conventional spectrometer using simple optics. The measurement takes less than 1 min to perform, requires no sample preparation, and can be made through overlayers of non-lead-containing paint. The limit of detection is 0.014% Pb in latex paint, on a dry weight basis, with relative sample standard deviations of 5-10%.

Prior to the 1940s, lead compounds were commonly used as blocking and coloring agents in interior and exterior house paints. However, in more recent years, it was established that exposure to lead in house paint can produce lead poisoning in young children. Subsequently, federal guidelines were established to set abatement limits for lead in paint on existing housing at 0.5% (w/w, dry weight basis).<sup>1</sup> Although the Consumer Products Safety Commission currently regulates lead in paint, with 0.06% (w/w, dry weight basis) Pb being defined as lead-containing paint,<sup>2</sup> leaded paint can still be found in older structures. These old lead-based paints mobilize as dust during renovations, leading to a potential for high exposures. Since abatement of lead-based paint is very expensive, it is desirable to develop techniques that can be used on-site to rapidly identify lead-containing paint on walls and trim.

The instruments most widely used for the analysis of paint in the field are portable X-ray fluorescence (XRF) spectrometers. These instruments use the radiation that accompanies a radioactive decay as an X-ray source. K-shell radiation from lead is excited by instruments with Cobalt-57 sources, and L-shell measurements can be performed using Cadmium-109 or Curium-244 sources. At least one commercial instrument is available that can be used to perform both measurements. Since the penetration depth of portable XRF instruments is usually not known (varying from several centimeters for those that use K-shell X-rays to <0.1

mm for those that use L-shell X-rays),<sup>3</sup> they are typically used for screening and are calibrated to provide a positive indication if lead exceeds 1 mg/cm<sup>2</sup>. XRF analysis is rapid, a typical exposure time being 5 s. However, it does suffer from substrate effects and attenuation of signals from overlayers of non-lead-containing paint. Also, the measurement volume is typically not known. XRF screening is confirmed by independent analysis, typically dissolution followed by ICP emission.<sup>4</sup>

Laser-induced breakdown spectroscopy (LIBS) is a useful method for determining the elemental composition of solids. This method has been recently reviewed, and a number of different applications have been described.<sup>5</sup> In the LIBS technique, a high-power pulsed laser is used to generate a plasma from the sample of interest. For example, a 10-ns pulse from a 100-mJ laser delivers a peak power of 10 MW. Furthermore, when focused to a 100- $\mu$ m-diameter spot, this pulse produces a peak power density of about 130 GW/cm<sup>2</sup>. At such high power densities, the electric field is strong enough ( $> 10^6$  V/cm) to produce dielectric breakdown of any medium. Plasmas consisting of atomic and ionized species can thus be produced from most matrices. This phenomenon was first demonstrated in 1963 using a pulsed ruby laser.<sup>6</sup> The elemental composition of the sample is accomplished by measuring the atomic emission from the atoms and ions in the plasma.

There are many reports of the use of LIBS for measuring metals in solid substrates.<sup>7-11</sup> There have also been LIBS investigations of aerosols,<sup>12</sup> single particles,<sup>13-15</sup> metals in solution,

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# Patterned Silver Nanorod Array Substrates for Surface-Enhanced Raman Scattering

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A novel method for batch fabrication of substrates for surface-enhanced Raman scattering (SERS) has been developed. A modified platen that fits in a commercial electron beam evaporator enables the simultaneous deposition of Ag nanorod arrays onto six microscope slides by glancing angle deposition. Following removal of substrates from the evaporator, patterned wells are formed by contact printing of a polymer onto the surface. Well dimensions are defined by penetration of the polymer into the nanorod array and subsequent photochemical curing. Inherent advantages of this method include: (1) simultaneous production of several nanorod array substrates with high structural uniformity, (2) physical isolation of nanorod arrays from one another to minimize cross contamination during sample loading, (3) dimensional compatibility of the patterned array with existing SERS microscope, (4) large SERS enhancement afforded by the nanorod array format, (5) small fluid volumes, and (6) ease of use for manual delivery of fluids to each element in the patterned array. In this article, the well-to-well, slide-to-slide, and batch-to-batch variability in physical characteristics and SERS response of substrates prepared via this method is critically examined.

**Index Headings:** Surface-enhanced Raman scattering; SERS; Surface-enhanced Raman spectroscopy; Nanostructured silver surface; Micro-contact printing; Glancing angle deposition; GLAD.

## INTRODUCTION

Since its discovery in the mid-1970s,<sup>1–3</sup> interest in surface-enhanced Raman scattering (SERS) has steadily grown.<sup>4–7</sup> It has become a powerful analytical tool for determining analyte concentration and characterizing the vibrational, rotational, and other low-frequency modes of molecules and molecular ensembles.<sup>8,9–10</sup> One impediment to its application in biology is the difficulty involved in manufacturing SERS substrates with reproducible performance characteristics.<sup>5,11,12</sup> Recently, substrates comprising silver nanorod arrays have been shown to be highly uniform in structure, easy to fabricate, and provide high signal enhancements.<sup>13–25</sup> These substrates show promise as solutions to the existing impediment to biosensor applications.<sup>6,26–30</sup>

The nanorod arrays have been fabricated by glancing angle deposition (GLAD).<sup>19,20,31–39</sup> GLAD is a physical vapor deposition technique that creates thin films consisting of columnar microstructures as a result of atomic shadowing.<sup>40–42</sup> The fabrication of SERS substrates with reproducible and reliable performance characteristics depends on precise control of empirical parameters including flux angle, deposition rate, substrate temperature, etc.<sup>43</sup> The angles used during GLAD play a crucial role in both the physical nature of the microstructures and the porosity of the array.<sup>34,44,45</sup> Dense arrays are formed at low flux angles, whereas porous arrays are formed at flux angles greater than 80°. For silver nanorod

arrays, a flux angle of 86° has been shown to be optimum for SERS applications.<sup>19–22,44–47</sup> Variation in the levels of this and other empirical parameters negatively impacts the consistency of the physical characteristics and SERS performance of substrates fabricated by physical vapor deposition. One solution to this problem is to prepare multiple substrates per batch and to pattern each substrate, enabling multiple analyses per substrate.

We present herein an improved method for batch fabrication and patterning of SERS substrates. We have designed a modified platen for a commercial electron beam evaporator that enables the simultaneous deposition of Ag nanorod arrays onto six microscope slide substrates. Following removal of slides from the evaporator, wells are patterned by contact printing of a polymer onto the nanorod array surface. Well dimensions are defined by penetration of the polymer into the nanorod array and subsequent photocuring. Inherent advantages of this method include: (1) simultaneous production of several nanorod array substrates with high structural uniformity, (2) physical isolation of nanorod arrays from one another to minimize cross contamination during sample loading, (3) dimensional compatibility of the patterned array with existing SERS microscopes, (4) large SERS enhancement afforded by the nanorod array format, (5) small fluid volumes, and (6) ease of use for manual delivery of fluids to each element in the patterned array. In this report, the well-to-well, slide-to-slide, and batch-to-batch variability in physical characteristics and SERS response are critically examined.

## EXPERIMENTAL

**Substrate Fabrication.** The SERS substrates were fabricated from standard 25 × 75 mm glass microscope slides. The process began by immersing each slide in "piranha" solution for 30 min, copious rinsing with deionized water, and drying under a flowing nitrogen gas stream. Next, slides were placed in a CVC-601 DC Sputtering tool (Consolidated Vacuum Corp., Rochester, NY) and thin films of titanium (50 nm) followed by silver (500 nm) were deposited onto one side of the slide normal to the surface. The titanium underlayer promotes adhesion of the silver to the glass surface. Then, the substrates were positioned on the specially designed sample stage in the CVC-SC5000 electron beam evaporator (Consolidated Vacuum Corp., Rochester, NY). Silver (99.99% from Kurt J. Lesker, Clairton, PA) was deposited onto the slides at a deposition rate of 3 Å/s, a starting pressure of 1 × 10<sup>-6</sup>, and at a flux angle of 86° from normal until the apparent deposition thickness on the quartz crystal thickness monitor reached a deposition thickness reading of 1.5 μm. Since the monitor is located closer to the source metal, this value is not representative of nanorod length or average film thickness. The substrates were allowed to cool under vacuum for at least 10 min prior to backfilling the chamber with nitrogen and

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## Plastic Tip Arrays for Force Spectroscopy

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The mechanical stability and viability of molecules investigated with the atomic force microscope (AFM) continue to be limiting factors in the duration of force spectroscopy measurements. In an effort to circumvent this problem, we have fabricated an all-plastic array of over 30 000 tips with dimensions similar to common AFM probes using silicon micromolding techniques. This approach enables rapid fabrication of tip arrays with improved properties, as compared to tip arrays made entirely of silicon.

The invention of the atomic force microscope<sup>1</sup> (AFM) has led to a revolution in imaging and characterization of biomolecules. Submolecular-scale images of biomolecules and biomolecular assemblies can now be obtained in air, in a vacuum, or in liquid. For example, Bustamante and co-workers<sup>2</sup> obtained high-resolution images of the diffusion of *Escherichia coli* RNA polymerase along DNA. Their results provided new insight into the mechanism by which RNA polymerase searches for the promoter, a key step in transcription.

In force spectroscopy, an AFM is used as a surface forces apparatus, and mechanical measurements on single molecules are made. In these measurements, a single molecule is attached to both the probe tip and an opposing surface. A tensile force is applied to the molecule by retraction of the surface by the piezo scanner. Simultaneous monitoring of cantilever deflection affords measurement of the molecule's response to the applied load. The mechanical properties of titin and other proteins,<sup>3–6</sup> DNA,<sup>7–9</sup> polysaccharides,<sup>10–12</sup> and several polymers<sup>13,14</sup> have been determined using this approach.

Performing repetitive measurements with conventional probes remains problematic. Oftentimes, force spectroscopic experiments are cut short because immobilized molecules are damaged or lose activity.<sup>15</sup> Mechanical or chemical degradation of molecules on the tip requires the exchange of the AFM probe and, therefore, exposure of the substrate to the environment. Green and co-workers<sup>16</sup> utilized tip arrays in conjunction with tipless cantilevers to circumvent this problem. When a tip loses functionality, a simple translation of the cantilever to a new tip in the array permits experiments to continue. Since the contact area of the tip is small, the number of possible fresh interaction sites with a tipless cantilever is very large.

The tip array fabrication method used by Green et al. was subtractive.<sup>16</sup> Briefly, a 200–250-nm-thick protective layer of oxide was grown on a silicon (100) wafer and lithographically patterned. The tips were created with an anisotropic etch that undercut the oxide caps. Repetitive microscopic examination of tips during processing was required to attain the desired shape.

In this Technical Note, we present a method for fabricating tip arrays out of the photodefinable epoxy SU-8. Our method, schematically illustrated in Figure 1, utilizes silicon micromolding techniques to define the shape of the tip; arrays of sharp tips are produced in a single step.

SU-8 when cured is chemically inert; resistant to solvents, acids, and bases; mechanically robust; and optically transparent. These properties render SU-8 a suitable material for tip array applications. When compared with tip arrays made from silicon, arrays fabricated from SU-8 require fewer processing steps and cost less per array. In addition, literally hundreds of tip arrays can be produced from a single mold.

### EXPERIMENTAL SECTION

The fabrication procedure is as follows: SiO<sub>2</sub> or Si<sub>3</sub>N<sub>4</sub> was thermally grown (over 1 μm thick) onto the polished surface of a single crystal Si (100) wafer. A thin film of negative tone photoresist (Shipley 1827) was applied, and an array of squares was lithographically patterned onto the resist (Karl Suss MA-6 Mask Aligner). Following development, anisotropic etching preferentially along the {111} planes of the exposed Si surface in aqueous KOH (Fisher Scientific, ACS Grade) (10 wt % in water, 65 °C, 30 min) yielded square pyramidal voids in the silicon wafer. These voids served as the mold for defining the shape of the plastic tips. Nano SU-8 photoresist (Formulation 25, MicroChem

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## Review

### Mammaglobin: a candidate diagnostic marker for breast cancer

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#### Abstract

Mammaglobin, known for its mammary tissue specificity, has been discussed as a promising diagnostic marker in breast cancer for almost 10 years. In particular, the application of mammaglobin RT-PCR to detect disseminated breast cancer cells has been reported. More than 25 publications evaluate the detection of mammaglobin mRNA in lymph node, blood, and bone marrow specimens of breast cancer patients. Recently, structural details about the mammaglobin complex have been discovered, and these findings can be implemented to optimize detection of the secreted protein. This review summarizes the findings of almost 50 published studies and the current knowledge about the diagnostic utility of mammaglobin.

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**Keywords:** Mammaglobin; Secretoglobin; Breast cancer; Diagnostic marker; RT-PCR; Serum

#### Introduction

Human mammaglobin (h-MAM) was identified by Watson and Fleming [1,2] in 1996 using differential display PCR technique. Mammaglobin belongs to the uteroglobin/Clara cell protein family of small epithelial, secretory proteins, which has recently been named secretoglobins with currently 23 known family members [3,4]. The founding family member, uteroglobin, was discovered as the major protein component of rabbit uterine secretions [5] and was the first progesterone-regulated mammalian protein discovered. All six human member genes are localized on chromosome 11q12.2 and form a dense cluster [4]. Mammaglobin has been found in breast epithelial cells and is overexpressed in breast cancer [2]. Due to its breast-specific expression, mammaglobin mRNA and protein are of high interest as candidate diagnostic markers for breast cancer. With over 50 publications on its use since the year 2000, it is considered the most promising molecular marker for breast cancer to date.

#### Expression in primary tumors

Mammaglobin expression was first studied by Watson and Fleming [2] in 35 breast carcinomas using RT-PCR and Northern blot analysis. They reported overexpression of mammaglobin (at least 10-fold relative to normal breast tissue by Northern blot hybridization) in 23% of the tumors tested with no correlation to a specific histology. In a subsequent study, no gene amplification or gene rearrangement was found in tumors overexpressing mammaglobin [6], indicating changes in transcriptional regulation. The same group later studied mammaglobin protein expression by immunohistochemistry and detected strong reactivity in 81 out of 100 breast tumors [7] independent of stage and histological type of the cancers. In this study, mammaglobin mRNA was found for the first time in lymph nodes demonstrating the utility of mammaglobin-directed RT-PCR for detection of disseminated tumor cells. Leygue et al. [14] found mammaglobin expression in tumor epithelial cells, but not in stromal or inflammatory cells, analyzing 13 breast tumor tissues using *in situ* hybridization.

Recently, Nunez-Villar et al. [8] evaluated an extensive panel of 128 breast cancer specimens by RT-PCR. A correlation between high levels of mammaglobin and expression of estrogen and progesterone receptor, diploid DNA content, low Ki67 labeling index, low nuclear

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**C. Introduction (*one paragraph for objectives; one paragraph for each technique*)**

Explain the objective(s) of the experiment and specify the instrumental technique(s) used for the analysis. Briefly describe the theory behind the instrumental technique and identify the essential components of the instrument (a schematic diagram is preferred).

**D. Experimental Section (*two paragraphs*)**

***Methods.*** This paragraph should simply reference the web page where the experimental protocol is given. Delineate any changes or deviations from that referenced protocol that were made.

***Instrumentation.*** This paragraph should identify all equipment used (manufacturer, model number, city and state where manufacturer is located).

## E. Results

This section should include all your raw data, data tables, pertinent results tables, graphs, balanced chemical equations, sample calculations with the correct number of significant figures and a numerical error analysis.

*Raw Data.* For the raw data, you have the option of providing this data in tabular form (typed).

*Graphs.* Graphs should be constructed using computer graphics or spreadsheet programs and should have the equations for any least-square fitted curves printed on the plot. Each plot should have a figure number and title, and the abscissa and ordinate should have labels with units. Each graph should either have a legend or a figure caption that clearly states what the graph presents.

*Tables.* Each table should have a Roman numeral and title, and each row and column should have a heading with units if appropriate. The number of significant figures presented in the tables should be consistent with the number of significant figures given for your results reported in previous sections.

## **E. Results (cont'd)**

*Sample Calculations (for ease of grading).* Using one set of representative data and starting from the numbers on your experimental data sheet, show all pertinent steps in the calculation made to obtain the numbers reported in your intermediate calculation tables. Include calculations using regression lines from plots. Give formulas used, and show substitution of numbers (with units and proper number of significant figures) into formulas. Give sample calculations for literature values which you may have calculated.

## **E. Results (cont'd)**

*Sample Calculations (for ease of grading).* Using one set of representative data and starting from the numbers on your experimental data sheet, show all pertinent steps in the calculation made to obtain the numbers reported in your intermediate calculation tables. Include calculations using regression lines from plots. Give formulas used, and show substitution of numbers (with units and proper number of significant figures) into formulas. Give sample calculations for literature values which you may have calculated.

## F. Discussion

Briefly discuss the significance of the results, and their relationship to the theory behind the method. Are the results what you expected? Why or why not? A discussion section should include:

*Statistical Analysis:* A brief discussion of the accuracy and precision and statistical errors in your results is appropriate.

*Analysis of Sources of Error:* Discuss the sources of error in this experiment and in the instrument. Classify these sources; identify whether they result from the skills of the experimenter, the quality of the instrument, or are intrinsic to the measurement. Suggest ways to reduce their impact on the final determination.

*Significance:* What is the significance of your data and results? How do they relate to information already known about the 'real' or 'exact' values? Also, any time you exclude raw data from your results, you should discuss why. A discussion of what these results mean is also appropriate (going back to the importance of the goals listed in your abstract).

## **F. Discussion (cont'd)**

*Conclusion:* Briefly have a conclusion that addresses the goals laid out in your abstract. Numerical results should be reported, along with the uncertainties ( $\pm$  std dev. or  $\pm$  r.s.d.) associated with them. Identify how the precision of repeated determinations compares with the uncertainty in the final result as determined by the propagation of error analysis.

## **G. Points for Discussion**

Provide answers to questions in the discussion section. If the answer involves calculations, do not just put down the number. Answer in complete sentences.

## **H. References Cited**

Cite your sources of information. Consecutively number each within the body of your report and list them at the end. Use the formatting found in the journal *Analytical Chemistry*.