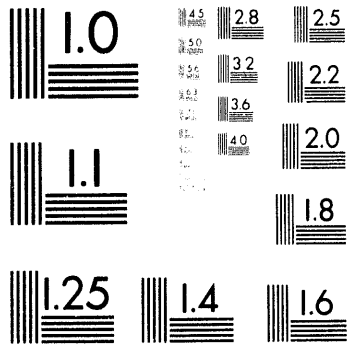
1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
6.3

1 of 1

DOE/ER/13996--4

Michael D. Morris

ER13996

Progress Report, 5/1/92-6/30/93

A. Confocal Raman microscopy: We have developed digital confocal Raman microscopy (1). In this 3-dimensional technique, a stack of Raman images is taken at intervals of 0.1-2 microns through the depth of the sample. The point spread function of the microscope is then deconvolved from the images, to yield a stack of sharply depth-resolved images. A constrained iterative deconvolution, which is computationally expensive, is used. The technique avoids the decrease of signal/noise ratio which accompanies the simpler nearest-neighbor deblurring and yields images which are equivalent to or better than obtainable with a confocal microscope. The technique efficiently uses the available laser power and makes confocal Raman imaging possible. The procedure has been tested on polystyrene beads and shown to work well. The computation time has recently been reduced from about 45 minutes to about 2 minutes, using a digital signal processor (DSP) instead of the CPU of the general purpose workstation previously employed. We expect that increasingly fast and inexpensive DSP's will reduce the time to under a minute in the near future.

In collaboration with a major glass maker, we have recently employed confocal Raman microprobe spectroscopy and imaging to identify and image molecular sulfur inclusions in glass pellets. Sulfur is formed by the decomposition of potassium sulfate, a constituent of many glasses. On the microscopic scale, we have been able to estimate sulfur film thickness at about 1 micron in inclusions of 20-30 micron diameter. The gaseous component of the inclusions has been tentatively identified as SO_2. Analysis of this data is still in progress.

B. Raman spectroscopy and imaging of electrophoretic systems: We have used the Raman spectrum of water as a non-invasive temperature probe in operating electrophoresis capillaries (2). Briefly, the experiment uses the temperature dependence of the equilibria among various hydrogen-bonded forms of water, as measured by changes in the OH-stretching region (ca. 3300 cm^{-1}). The technique is simple, fast (<3 sec in recent measurements) and capable of micron spatial resolution. We have demonstrated that existing theories of capillary operating temperature are inadequate, because they assume an isothermal capillary. Longitudinal temperature gradients exist, because portions of the capillary are heat-sinked through mechanical supports or immersion in the buffer reservoirs at either end. Recently, we have demonstrated that steady state is reached rapidly (<30 sec) and that in the absence of active cooling, the temperature is uniform across the radius of the capillary at most points along its length.

C. Macro-scale Raman imaging: A very simple macro-scale imager has been constructed. It consists of our CCD camera and a C-mount video camera lens. The system is operated in a close-up mode with a field of view of 25-100 mm, depending on the sample at hand. One or two holographic notch filters and an interference filter are used to isolate the Raman scatter. The interference filter will be replaced with a two-stage liquid crystal Fabry-Perot interferometer by mid-August. We have demonstrated that the system can image water Raman scattering using 30 mW 532 nm, and can be used to map boundaries and impurity distributions and morphological changes in polymers and ceramics. With NIR excitation, it may also be useful for some clinical diagnostics.

D. Raman microprobe instrumentation: We continued our explorations of holographic optical elements (3). A holographic beam splitter, essentially a tilted notch filter, was constructed to our specifications by Kaiser Optical Systems (Ann Arbor). The high efficiency filter replaces the conventional 50/50 beam splitter used in Raman

microprobes. It injects 90% of the laser light and passes 75-80% of the Raman scatter, for a 3-fold gain in collected signal. With it, we have been able to use low power lasers to obtain Raman microprobe spectra and Raman images as close as 50 cm^{-1} from the exciting line.

E. Surface-enhanced Raman Spectroscopy: To obtain micron-resolved surface-enhanced Raman spectra (SERS) we have devised a micron-diameter silver probe (4). The device consists of a silver wire micromachined to a diameter of 0.5-5 microns by anodization against a platinum ring electrode. The probe has been used to obtain spectra from inside zebra fish embryos at various stages of development from 20 minutes to 10 hours after spawning. The spectra have not been completely interpreted. Spectra of retinoic acid, another carotenoid and several amino acids have been identified and their time-course charted. The carotenoids, which originate in the yolk, are differentially consumed as the embryo grows. Zebra fish embryos, which are readily available from the U. of Michigan biology department, are a model system only. It is intended to operate this device as a neurochemical probe, because it can detect GABA and histamine at release levels and quite possibly at resting levels as well.

Refereed Publications resulting from this work: (in order of citation)

1. Govil, Anurag; Pallister, David M.; Morris, Michael D., Three-dimensional Digital Confocal Raman Microscopy, *Appl. Spectrosc.* **1993,** *47,* 75-79.

2. Davis, Kevin L.; Liu, Kei-Lee; Lanan, Maureen; Morris, Michael D., Spatially Resolved Temperature Measurements in Electrophoresis Capillaries, by Raman Thermometry, *Anal. Chem.* **1993,** *65,* 293-298.

3. Pallister, David M.; Liu, Kei-Lee; Govil, Anurag; Morris, Michael D.; Owen, Harry; Harrison, Timothy R., A Raman Microprobe with Holographic Beam Splitter for Low Frequency Operation, *Appl. Spectrosc.* **1992,** *46,* 1469-1473.

4. Todd, Elizabeth A.; Morris, Michael D., Fabrication of Micron Diameter Silver Electrodes for Surface-Enhanced Raman Spectroscopy, *Appl. Spectrosc.* **1993,** *47,* 875-877.

Work Plan 11/15/93-11/14/94

With the installation of our liquid crystal interferometric filter the conversion of our system to efficient direct Raman imaging will be complete. Our goals are to explore some of the applications of Raman imaging which we have previously proposed and to carry out some exploratory projects which will form the basis for subsequent proposals. Both microscopic and macroscopic imaging will be used.

A. Fiberglass/epoxy composites: We will undertake our proposed studies on fiber-reinforced plastics. Various defects produced during the molding process significantly effect the composite's mechanical properties. Of these defects, voids are considered to be the most critical in influencing composite performance and often occur at the matrix resin / fiber interface and is proposed to be related to heterogeneous distribution of components and uneven wetting. We will use micro Raman imaging fto map the distribution of binder and sizing on E-glass fibers. Chemical inhomogeneities and interfacial regions near the surface of molded composites can be mapped using our 3-D technique. To simplify the

experiments, we will choose mats where the binder and sizing are chemically quite different.

Our initial experiments will map distribution of sizing components on epoxy resin-compatible E-glass fibers. Typical sizings consist of bisphenol-A-based epoxy resin / aliphatic polyether, along with a silane for covalent bonding. The phenolic moieties of the epoxy resin are para-substituted benzene rings and can be Raman-mapped in by their strong ring-stretches at ca. 1600 cm^{-1} or alternatively by the strong aromatic C-H stretch at ca. 3050 cm^{-1}. The polyether lubricant is more of a challenge, because it will resemble the hydrocarbon backbone of the epoxy resin, spectroscopically. Candidate bands for imaging such as the C-O stretch (ca. 1100 cm^{-1}) or the CH2 wag (ca. 1450 cm^{-1}) or even C-H stretch (ca. 2900 cm^{-1}) would be adequately characteristic at high resolution (3-6 cm^{-1}), as used in microprobe spectroscopy. However, there will be substantial overlap in the images, where the spectral resolution will be 20-30 cm^{-1}. Thus, principal components analysis will be necessary to separate binder images from polyether images. Assuming a relatively uniform coating, we expect to encounter sizing coating thickness of 3-10 μm. The composite can have variable thicknesses, but simple sectioning can provide a sample suitable for study. Three-dimensional imaging will be necessary, particularly in the region of voids. Because depth profiling is needed, digital confocal imaging will probably be faster than conventional confocal imaging. However, in the crucial region around voids, where large abrupt (air/organic) refractive index changes are encountered, image restoration may fail. In that case, the slower common path confocal system can be used.

We will use three-dimensional Raman mapping to measure sizing homogeneity in regions well removed (>2 mm) from any visible defect and in regions close to voids. If stratification is observed only near voids, or is greater there, then it may be the cause of poor adherence. Conversely, if stratification is observed everywhere, then it can not be correlated with coating quality.

A second hypothesis is that formation of a void may be correlated with a local change in chemical structure near the void region, perhaps caused by reaction with an adventitious impurity. If there is no clear stratification or other inhomogeneity until quite close to a void boundary, then the impurity hypothesis must be investigated. Here, the simplest probe would be confocal Raman microprobe spectroscopy. We are aware of the limitations of Raman and infrared spectroscopy for qualitative analysis of mixtures. However, local content of perhaps 10% or higher of an adventitious compound should be observable by confocal Raman microprobe spectroscopy, in a volume element of about 1-5 μm^3.

B. Raman imaging thermometry: We are now ready to map temperatures in electrophoretic gels. In a modest departure from our original proposal, we plan to use a hybrid Raman/fluorescence approach. We will use the temperature dependence of the pKa of fluorescein or another dye in blank gels to map global temperatures. This approach is adequate for measuring heat transport properties and cooling system effectiveness. However, such dyes intercalate with nucleic acids and generally perturb electrolyte distributions in the vicinity of a nucleic acid band. Therefore, water Raman macro-imaging, using a field of view of 15-25 mm, is preferred for temperature gradient measurements in the regions of nucleic acid content. These measurements will indirectly measure electrolyte distribution (i.e., Joule heating), in the band regions. Nucleic acid concentrations are not negligible compared to buffer ion concentrations. Consequently, we expect to find gradients of several degrees across a band. From these measurements

we can calculate the expected band broadening caused directly by temperature and by electrolyte-induced perturbations to effective mobilities. The results should be especially useful in interpretation mobility patterns in pulsed field gel electrophoresis. Isothermal and isoconductance conditions are usually assumed, and there is general agreement that theories are qualitatively correct, but quantitatively inadequate.

C: Neurochemical SERS: With a proven system for intracellular SERS measurements, we are ready to undertake preliminary SERS measurements in neurochemical matrices. Our first targets will be simple microdialysis systems, where 20-30 micron resolution is probably adequate, but where simple techniques for histamine and GABA, two known SERS-active neurotransmitters, are lacking. GABA is the major inhibitory neurotransmitter. Histamine is important, because it is released primarily in the hypothalamus, an organ which controls reproductive behavior (among other things) and whose morphology is putatively related to sexual orientation. We will then move to rat brain slices, where direct measurements have never even been attempted. The uses of a micro-electrode probe are not limited to neurochemistry, of course. The device is well-suited to probing chemical composition in a variety of biological structures and has potential applications to a wide-variety of health-related problems of more direct interest to DOE.

The graduate student working on this project is supported by fellowships from The University of Michigan and The American Chemical Society. DOE-funded equipment will be employed and DOE funds will be used for incidental operating expenses.

DATE FILMED

1/3/94

END