DOE/ER/13996--T1

HIGH DEFINITION RAMAN IMAGING

Final Report

for Period May 1, 1992 - November 14, 1995

Michael D. Morris

University of Michigan
Ann Arbor, Michigan 48109-1055

May 1996

Prepared for THE U.S. DEPARTMENT OF ENERGY
AWARD NO. DE-FG02-89ER13996

DISCLAIMER

Final Report, 5/1/92-11/14/95

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 used on a number of polymeric samples, including polystyrene beads and polyester gratings, 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 potassium sulfate and 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. Using imaging, we have studied distribution of potassium sulfate crystals and partially reduced sulfur salts, tentatively identified as potassium thiosulfate has been studied. The gaseous component of the inclusions has been tentatively identified as SO_2. For this work we have employed He-Ne laser excitation and anti-Stokes imaging, which effectively solve the fluorescence problem. Since the strong Raman bands of the sulfur species all lie below 500 cm^{-1}, the loss of intensity on anti-Stokes imaging is not serious.
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 (2,3). 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. However, longitudinal temperature gradients exist, because portions

of the capillary are heat-sinked through mechanical supports or immersion in the buffer reservoirs at either end. The consequence is band broadening, which can degrade ultrahigh resolution separations, although it is not important for routine work. We have demonstrated that steady state is reached rapidly (<30 sec). This finding also contradicts literature models because of the unrealistic boundary conditions used. We have shown, however, that in the absence of active cooling, the temperature is uniform (<2°C) across the diameter of the capillary at most points along its length. Here, our findings are in agreement with the classical heat transport calculations. The reason is that while the conventional longitudinal boundary conditions are unrealistic, the much simpler radial boundary conditions are realistic. Consequently, heat transport predictions are accurate.

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. Illumination is provided by 3 or 4 10 mW He-Ne lasers, arranged as in a photographic copy stand. The system is operated with a field of view of approximately 25-30 mm, and a working distance of 25-30 cm, depending on the sample at hand. One or two holographic notch filters and an angle-tuned 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 late October. We have demonstrated that the system can image water Raman scattering using 30 mW 532 nm, and can be used to map boundaries in vegetable fibers 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 (4). 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.

Faster tuning is available from a liquid crystal tunable filter. A device built to our specifications provides 11 cm^{-1} band width and 15-50% throughput (5,6). It consists of two liquid crystal Fabry-Perot interferometers in series. One sets the resolution and the other is an order-sorter.

We have described the effects of Koehler and non-Koehler illumination on Raman images taken with a wide-field microscope (7). Contrast and resolution are best under Koehler conditions, which have not always been used in Raman microscopy.

E. Surface-enhanced Raman Spectroscopy: To obtain micron-resolved surface-enhanced Raman spectra (SERS) we have devised a micron-diameter silver probe (8). 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. Spectra of retinoic acid, another carotenoid and several nucleosides have been identified and their time-course charted (9). 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. At the same time, we have shown that resonance-enhanced surface-enhanced Raman spectra can be used to detect nanomolar levels of dopamine (10). Dopamine is converted to its ferric catecholate complex (λ_{max}=470 nm). The complex gives strong SERS under 532 nm excitation, and the 1480 cm^{-1} band is characteristic for dopamine.

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. Liu, Kei-Lee; Davis, Kevin L.; Morris, Michael D., Raman Spectroscopic Measurements of Spatial and Temporal Gradients in Operating Electrophoresis Capillaries, *Anal. Chem.* **1994,** *66,* 3744-3750
4. 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.
5. Morris, Michael D., Ultrasensitive Raman and fluorescence imaging using liquid crystal tunable filters, *Proc. SPIE* **1995,** *2385,* 80-88.
6. Christensen, Kenneth A.; Bradley, Nancy L.; Morris, Michael D.; Morrison, Robert V., Raman Imaging using a Tunable Dual-Stage Liquid Crystal Fabry-Perot Interferometer, *Appl. Spec.* **1995,** *49,* 1120-1125.

7. Pallister, David M.; Morris, Michael D., Laser Koehler epi-illumination for Raman and fluorescence Microscopic Imaging, *Appl. Spectrosc.* **1994,***48,* 1277-1281.

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

9. Todd, Elizabeth A.; Morris, Michael D., Micron Surface-Enhanced Raman Spectroscopy of Intact Biological Organisms and Model Systems, *Appl. Spectrosc.* **1994**, *48*, 545-548.

10. Kowalchyk, Will K.; Davis, Kevin L.; Morris, Michael D., Surface-enhanced Resonance Raman Spectroscopy of Iron-Dopamine Complexes, *Spectrochim. Acta* **1995**, *51A,* 145-151.