SPECTROMICROSCOPY**Final Report**

CSP No. 94-LANL-050-D2

A. Parties

This project is a relationship between Los Alamos National Laboratory, P. O. Box 1663, Los Alamos, NM 87545 and:

ERIM International, Inc.
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B. Project Scope

The objectives of this project were: (1) to develop a Confocal Hyperspectral Imaging Raman Microscope (CHIRM), and (2) using modern image processing technology, to devise algorithms for hyperspectral microscopic image recovery and enhancement. The first objective involved the development of advanced techniques in confocal microscopy, spectroscopic image detection, and image processing to produce hyperspectral Raman images. The second objective was to exploit the image detectors together with advanced statistical and data handling techniques, to produce enhanced Raman spectral images and to extract the maximum amount of spectral information in the minimum time with maximum economy. In both cases the objective was to develop instrumentation and software for both diagnostic and research applications, which require maximum sensitivity and minimum complexity and cost. We have completed these objectives on time and with a reduced budget.

The CHIRM instrument we have developed combines the information on molecular structure from Raman scattering (e.g. the vibrational fingerprint region) with ultimate spatial resolution (sub-micron) to produce images that reveal the location, distribution, and structure of specific molecules in the materials of interest. We have demonstrated numerous applications for such powerful and specific diagnostic capabilities, from weapons process stream monitoring and materials characterization to biomedical research. CHIRM can be applied to the surveillance of vital structural details which determine materials properties and ultimately device performance, for example the specific changes in molecular structure that accompany aging of a high explosive formulation on the length-scale of the grain boundaries of the explosive and binder.

The original focus of this project was to develop a new optical imaging tool (CHIRM) for biological and health applications. Approximately midway through the life of this project, the focus was redirected to apply this new technology to weapons related materials issues, such as new materials development and characterization and the aging of materials critical to weapons reliability and performance. Raman microscopy and imaging (and the complimentary fluorescence spectra and imaging available with our CHIRM system) provide data at the molecular level through the resulting spectra and images. This approach then allows for information on the underlying chemistry in this processing. A fundamental understanding of the chemistry is essential for modeling and improving the product reliability, certification and control. We have obtained preliminary data which suggests that the technology we are developing with our TTI project is likely to provide important new insight into fundamental, weapons-related materials issues. In addition, our industrial partners gave their enthusiastic support of this redirected application of the technology that we jointly developed.

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C. Technical

1. Project tasks and progress.

- Task 1: project management/documentation
- Task 2: develop image processing software
- Task 3: hyperspectral image analysis
- Task 4: optical hardware development
- Task 5: develop stand-alone software packages
- Task 6: integrated testing
- Task 7: final report and closeout

Task 5 was not pursued to significant budget cuts in the project. However, all of the other tasks were completed, despite a major redirection of the focus of the project approximately midway through the life of the project.

2. Project technical accomplishments.

In the last several years microscopic Raman techniques have matured dramatically. Advances in detector, spectrograph and Rayleigh filter technologies have allowed the construction of robust, compact, high throughput micro-Raman systems. These systems have found applications in diverse areas¹ from ceramics^{2,3} and polymers⁴⁻⁷ to medicine^{8,9} and pigment analysis of fine art^{10,11}. Most of the applications detailed to date use a laser beam focused to a small spot on the sample (point illumination). The scattered light is collected and dispersed, then detected using either a linear multi-element array or a CCD camera. In this way a highly resolved Raman spectrum can be obtained and, with the use of confocal optics, spectra can be obtained from very small volumes of sample ($<1 \mu\text{m}^3$). By moving the sample, a 2D grid of spectra can be obtained. Raman spectral images are generated by plotting the intensity of the Raman band or bands of interest at each sample position. The information that can be obtained using a confocal, micro-Raman system is 4 dimensional (3 spatial and one spectral). Almost all modern micro-Raman systems utilize sensitive CCD cameras so that 2 dimensions of the available 4 dimensional information can be collected simultaneously. Some type of scanning is required to collect data in the remaining dimensions. The difference between the available micro-Raman systems is usually associated with the dimensions which are scanned.

Another approach to Raman imaging has been developed which probes two spatial dimensions at a single frequency. In this approach the sample is illuminated globally but usually not uniformly. Some sort of filter is used to select a single frequency and a 2D detector is used to form an image at this frequency. In the past, spectral resolution has somewhat limited the utility of this technique, however, recent advances in tunable, liquid crystal filter technology look promising¹². It is not possible to use this direct Raman imaging approach in a confocal mode so the data that can be obtained is essentially only three dimensional, with the possibility that out of focus scattered light may obscure or blur the Raman images. In the near future, however, we expect it will be possible to generate confocal images without the confocal aperture, using newly developed algorithms that operate on stacks of "blurred" images taken at different depth positions (see Shaw¹³ and Holmes et al.¹⁴, and references therein). Another possibility may be to generate a "virtual" aperture using a CCD camera¹⁵.

The point focusing approach outlined above, when operated in a confocal geometry, can give well resolved 4D information. However, this approach does not utilize the 2D capabilities of a CCD camera. As a result it is an extremely, often impractical, time consuming approach. A superior approach is to use either an optical scanner or a cylindrical lens to image the laser beam to a line on the sample. This line can then be imaged onto the entrance slit of a stigmatic, imaging spectrograph, attached to a CCD camera. The 2D images obtained contain one spatial and one spectral dimension. To obtain 2D spatial information the sample is moved in one dimension perpendicular to the length of the scanned laser line (line mapping). There are at least two additional advantages of the line scanning approach: Firstly the spectrograph slit acts as a spatial filter, providing some confocal capability. Secondly, heating effects, resulting in sample damage, are reduced, compared to point illumination. Imaging the scanned laser line onto the slits of a spectrograph maximizes the illuminated area. For a given average incident power the amount of scattered light will be the same in both point and line illumination modes, but in line illumination mode the average power can be increased without optical damage because the power density is substantially decreased. Ivanda and Furic¹⁶ found for their experimental setup that for the same excitation power density in point and line illumination, line illumination gave a factor of 18 improvement (depending on the length of the scanned laser line) in signal to noise ratio over point illumination.

Several authors have considered line imaging: Ivanda and Furic¹⁶ analyzed a line focusing system, which utilized a cylindrical lens. Their instrumentation did not, however, preserve spatial information along the line. Drumm and Morris¹⁷ used a cylindrical lens for line focusing while Bowden et al¹⁸ used a scanning lens. Viers et al^{19, 20} also used a cylindrical lens, with an imaging photomultiplier tube to obtain spatially resolved spectra. Of the two approaches to line imaging (cylindrical lens or laser scanning) laser scanning is the best choice for two reasons. Firstly, when using a cylindrical lens to generate a line, the intensity distribution along the line will be asymmetric or Gaussian. Secondly, if scanners are used it is possible to make the system truly confocal^{21, 22}.

We have developed a confocal, hyperspectral line imaging Raman microscope that can produce high resolution, spatially resolved spectra. We also describe techniques we have developed for producing two dimensional, spatially resolved, Raman (spectral) images. Figure 1 shows a schematic of the line imaging Raman microscope used here. The system is built around a Zeiss Axiovert TV microscope. Unless stated, the 514.5 nm line from a Spectra Physics 2045 argon ion laser was used for excitation. The excitation intensity at the sample was controlled using neutral density filters. The laser beam was scanned prior to entering the microscope using a Cambridge Technology optical scanner controlled by a Dana Exact, model 506 sweep/function generator. For point focusing the signal generator is simply switched off. The signal generator allows control of

the line scan frequency and amplitude. The length of the line at the sample depends on the objective lens. Two objective lenses were used in the work described here: Either a Zeiss, Achroplan, infinity corrected, 63X dry objective lens (NA=0.95) or a Zeiss LD epiplan, infinity corrected, 20X dry objective lens (NA=0.4). The focused laser line is ~ 130 or 40 mm in length when the 20X or the 63X objective is used, respectively. In the figure legends we will simply refer to the objectives as the 63X or 20X objective.

Figure 1. Schematic diagram of CHIRM instrument

The scattered light from the scanned excitation laser line is collected by the objective lens (180 degree backscatter geometry). A tube lens inside the microscope forms a primary image (which is located just outside the microscope) and then a second lens (L1 in fig 1) further magnifies and images the scanned line onto the entrance slit of a Kaiser HoloSpec f/2.2 imaging spectrograph. The total magnification depends on the focal length of lens L1. An external holographic notch filter (Kaiser supernotch plus) is used to reject the laser line. For all line imaging a 50 mm slit was used (for some of the spectra collected in point focus mode a 25 mm slit was used), resulting in a spectral resolution of $\sim 5.7 \text{ cm}^{-1}$. Spectral calibration is performed using either standard lamp lines or the Raman bands of toluene. Detection is accomplished with a Photometrics C210 CCD camera attached to the holoSpec (for some point focus applications we also used a 0.25 m Acton spectrograph). The camera has a prime grade 512x512 chip with very few "bad" pixels. The vertical dimension (parallel to the slit) on the CCD camera contains spatial information while the horizontal dimension contains spectral information.

One disadvantage of the holospec is that it has a fixed grating which is optimized only for a specific excitation wavelength (as are the coated optics). So for different excitation wavelengths a different spectrograph is required. Our spectrograph can image the 50-2480 cm^{-1} spectral region with 514.5 nm excitation (low frequency Stokes grating), assuming a 25 mm detector width. Our CCD camera is ~ 12 mm wide so to cover the full spectral range the CCD camera was translated perpendicular to the holospec optical axis.

Two dimensional spatial information is obtained by translating the sample in the direction perpendicular to the laser line using a Cell Robotics translation stage, which is accurate to 0.1 mm. A PowerMac 7100/80 computer is used to control data acquisition and all (serially interfaced) peripheral devices. In point focus mode the CCD is binned and a 3-point median filter is applied to the spectra (this effectively eliminates cosmic ray spikes) before presentation. In the line focusing work described here the data obtained consist of stacks of line images. All spectra and image stacks are collected and processed in an automated fashion using custom software generated in IPLab. In point illumination mode, the image of the point (actually the point is a diffraction limited spot (Airy disk) of diameter 0.66 mm for 514.5 nm excitation) on the CCD is a few pixels wide but spans the full length of the CCD (spectral dimension). When collecting spectra we can simply bin the CCD but in many cases, in order to reduce readout noise, to speed up processing time and to increase the confocal capabilities of the system¹⁵, we collect and bin only the narrow strip of pixels that contain the spectral information. Slightly different set-ups were used for point and line focusing although this was not strictly necessary. For point focusing a 50 mm achromatic lens was used to focus the Raman light to a point at the slit. For line focusing the focal length of the achromatic field lens (L1) could be varied depending on the desired magnification. In most circumstances we chose lens L1 such that the length of a ~0.5 mm spot would be imaged onto 2-4 pixels (46-92 mm in our CCD camera).

Application to Living Cells: Red Blood Cells As an example of the line imaging technique applied to living cells we have performed measurements on red blood cells (RBC's). RBC's are biconcave disks with a diameter of ~ 8 μm ²⁴. Much of the volume of RBC's is occupied by the oxygen storage and transport protein, hemoglobin, hence it is likely that any Raman spectra we obtain will be due to hemoglobin. In addition, we expect significant resonance enhancement of hemoglobin Raman bands because 514.5 nm is coincident with the β absorption band of the heme²⁵.

Figure 2. Bright field (white light) image showing the path of the scanned laser line across an individual red blood cell.

Figure 2 shows a bright field image obtained by white light illumination of a microscope slide containing fixed red blood cells. An individual cell is illuminated with the laser, which appears as a green (514.5 nm) line across an individual cell. The two-dimensional CHIRM image is shown in Figure 3. The vertical axis is the spatial (line) dimension, whereas the horizontal axis is the spectral dimension.

Figure 3. Two dimensional CHIRM image.

This image contains a great deal of information that must be extracted using image analysis software. Figure 4 shows the Raman spectrum extracted from this data.

Figure 4. Raman spectrum extracted from 2D CHIRM image of red blood cell.

The spectrum is clearly that of hemoglobin²⁵. The band near 1365 cm^{-1} depends on the oxygenation state of the hemoglobin and is typically 1355 cm^{-1} for the deoxy form, 1379 cm^{-1} for

the oxy form of hemoglobin. We have noted several factors that can influence the shape of the cell with concomitant changes in both the intensity and position of some of the Raman bands. This is beyond the scope of the present work, the purpose of which is simply to show the utility of the line scanning Raman system.

Figure 5 shows a line image obtained when the scanned laser beam traverses the middle of a RBC. Clearly there is a large decrease in intensity in the middle of the cell, consistent with its biconcave shape. The thickness of RBC's vary from $\sim 1.5 \mu\text{m}$ near the middle up to $\sim 1.8 \mu\text{m}$ near the periphery²⁴. This indicates that we are able to observe sub micron level changes in living cells.

Figure 5. Spatial (line) image extracted from 2D CHIRM image of red blood cell.

Clearly the Raman bands of hemoglobin can be used to form images of single cells. The shape of RBC's depend on the experimental conditions (and on various disease states) so it is possible to track not only molecular decomposition (by monitoring changes in the Raman spectra) but also where this decomposition occurs in the cell. In some cases we were unable to observe decreased intensity in the middle of the cell (as is shown here). Instead we observe almost uniform Raman intensity across the cell, indicating the cells are more spherical in shape. Measurements on RBC's, such as those described here, are likely to be of great value in studies related to blood storage. As stored blood ages, the hemoglobin in the cells undergoes chemical changes and decomposition. The details of these changes are not known precisely but it is certain that these changes will be accompanied by specific changes in the Raman spectra of the cellular hemoglobin. The use of Raman microscopy is therefore likely to be a useful probe of these cellular modifications.

The advantages of Raman imaging over other spectral imaging techniques such as fluorescence, are obvious, although much greater sensitivity is required using the Raman approach. In fluorescence microscopy, usually molecular tags or labels are used which attach to specific molecular components. The fluorescence from these tags is then used to image the molecular component. The process of tagging is generally complex and time consuming. It is often the case that these tags can damage or perturb the material they are supposed to report on, and this is especially true for biological material. In addition, the broadband nature of the fluorescence limits the number of

components that can be imaged at any one time. In contrast, no tags are required for Raman imaging in principle (although it is certainly feasible and Raman tags have been used successfully recently²⁶) and hence any potentially perturbative effects of the tags are removed. In addition, the vibrational technique is more selective of the type of molecules probed and will detect structural changes to which fluorescence spectra are insensitive. With Raman microscopy it is possible to also selectively image different components by resonance enhancement of specific components. In the study of single cells for example, the molecular species that will dominate the image (DNA, cytochromes, carotenoids, etc.) can be selected simply by varying the frequency of laser excitation. The high spatial resolution attainable using Raman microscopy (demonstrated here) should enable the study of subcellular components. With heme proteins, the use of certain visible excitation wavelengths will resonantly enhance scattering making the study of these proteins particularly attractive. Imaging of heme proteins in single cells is underway and recently studies on myeloperoxidase and cytochrome b have been presented²⁷.

Figure 6. Hyperspectral, multi-line composite Raman image of a polystyrene bead.

In addition to the obvious advantages of Raman spectroscopy, modern computer algorithms (which can combine several tens or even hundreds or thousands of spectral images to produce a hyperspectral image) and image enhancement techniques applied to spectral microscopy will likely

produce hyperspectral images that are far superior to that of images at single frequencies. The image shown in Figure 6 is a composite image of a polystyrene bead, obtained with multiple line scans through the bead. It is a hyperspectral image obtained as the sum of all of the individual Raman bands. The signal to noise ratio for each Raman band is different, so simply adding the band intensities together generally does not produce a composite image that is superior (both numerically and aesthetically) to all of the single frequency Raman images³⁰. More complex algorithms are required to utilize the information content inherent in large hyperspectral image stacks. Hyperspectral image analysis is still in its infancy but with the launch of various airborne spectral imagers such as AVIRIS (Airborne Visible/Infrared Imaging spectrometer) there has been a flurry of activity in the field²⁸⁻³¹. Important areas of research are 1/ the development of data reduction algorithms that can reduce or compress the huge volume of hyperspectral data without loss of critical information^{28, 29} and 2/ the development of image fusion algorithms that can combine several spectral images into a composite image that contains more detail and less noise than any single spectral image on its own^{30, 31}. Activities in the area of hyperspectral image analysis is likely to increase dramatically in the near future, partly because the next generation of airborne spectral imagers have been developed (for example, the high spectral imager (HSI)³² is expected to be launched soon³³) and partly because of the wide interest in, and applicability of spectral microscopies.

There are several areas in which Raman microscopic line imaging may be an extremely useful analytical tool. A significant problem facing the US DOE is contamination of soils at various production and testing facilities. Remediation of contaminated sites depends on speciation information. Information of the oxidation state and chemical form of contaminants is a necessary precursor for the utilization of remediation technologies. Raman microspectroscopy can be of great value in this area. Not only for species identification but also for monitoring decontamination technologies, and recently we have studied some uranium contaminated soil samples. Under bright field illumination we have found that it is almost impossible to visualize uranium complexes in contaminated soil samples. This is not the case using fluorescence techniques, however. Many actinide complexes exhibit strong fluorescence and can therefore be visualized directly. The Zeiss axiovert microscope is ideally suited for combined Raman/fluorescence studies. For fluorescence studies we illuminated (with light from the condenser lamp) the soil samples from one side using the normal condenser optics in combination with an appropriate excitation filter. This allowed visualization of the various actinide complexes in the sample which could then be translated to coincide with the laser excitation beam coming from the opposite direction. In this way we have been able to differentiate various compounds in soil samples. Again we expect the line imaging techniques developed here to be extremely useful for mapping species distribution in these samples.

3. References

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D. Partner Contributions

A unique combination of expertise and resources was required to meet these objectives. The required expertise and resources were provided by the partners of this cooperative agreement: at

Los Alamos, spectroscopy and development of advanced instrumentation, and at ERIM International Inc. development of advanced algorithms, software, and hardware for image processing and signal recovery, based on defense applications in satellite and radar imaging but transferable to microscopic imaging.

E. Documents/Reference List

1. "Hyperspectral Microscopic Raman Imaging," G. Hastings & R. B. Dyer *Applied Spectroscopy* 1997 (in press).

F. Acknowledgment

1. The Participant has reviewed the final report and concurs with the statements made therein;
2. The Participant agrees that any modifications or changes from the initial proposal were discussed and agreed to during the term of the project;
3. The Participant certifies that all reports either completed or in process are listed and all subject inventions and the associated intellectual property protection measures attributable to the project have been disclosed;
4. The Participant certifies that proprietary information has been returned or destroyed by Los Alamos National Laboratory.

Nancy David, ERIM Int'l, Inc. Date

R. Brian Dyer, LANL

Date

FINAL ABSTRACT

TITLE: Spectromicroscopy

NUMBER: CSP-94-LANL-050-DP

We have developed a prototype Confocal Hyperspectral Imaging Raman Microscope (CHIRM). The prototype consists of state of the art optical imaging hardware and integrated image acquisition and analysis software. We have tested this integrated instrument on a range of applications, including biomedical (red blood cell molecular imaging), weapons components characterization and novel molecular materials structure (artificial membranes). This new technology is clearly a highly versatile method for molecular specific imaging.

PROJECT ACCOMPLISHMENTS SUMMARY

Title: Spectromicroscopy

DOE TTI Number: 94-LANL-050-DP

Industrial Partner: ERIM International, Inc.

BACKGROUND

Every molecular species has vibrational frequencies that are characteristic of that molecule and no other. In a living cell, for example, the component nucleic acids, proteins, carbohydrates, lipids, and carotenoids each give rise to a characteristic Raman spectrum. Furthermore these frequencies may shift in response to perturbations, such as respiratory stress or the metabolic state of a cell. The vibrational spectra of the molecules are detected either by absorption of infrared light by the sample, or by inelastic (Raman) scattering of monochromatic light in the visible region.

This project developed a new approach to Raman spectroscopy, confocal hyperspectral imaging Raman microscopy (CHIRM) that exploits the molecular sensitivity and spatial resolution of CHIRM as a diagnostic and research tool. Hyperspectral imaging means that many separate images are obtained simultaneously, each using a different wavelength of light. Thus the entire Raman "fingerprint" is recorded in the hyperspectral images, with the ultimate spatial resolution provided by confocal microscopy, to produce microscopic images that reveal the location, distribution, and condition of specific molecules within the sample. This spectromicroscopy approach is similar in concept to fluorescence microscopy using dyes that attach themselves to specific target molecules, but there are two important differences. First, the potentially perturbing effect of the stain is absent. Secondly, the vibrational technique is much more selective as to type of molecule observed, perfectly general (all molecules have Raman spectra), and detects frequency changes to which the dyes may be insensitive.

The development of the CHIRM technique required the development and synthesis of two major technologies. First, the imaging hardware had to be developed to produce confocal, hyperspectral Raman images. This was accomplished using advances in confocal microscopy, spectroscopic image detectors, and holographic Raman filters. Second, image analysis methods and software had to be developed to enable image processing and enhancement. This was accomplished using the unique qualities of the spectroscopic image detectors together with advanced statistical and data handling techniques, to produce enhanced Raman images and thereby to extract the maximum amount of spectral information in the minimum time with maximum economy.

The key to the success of this effort was the combination of the skills and expertise of LANL in hardware and Raman instrument development and our industrial partner ERIM International, Inc. in image analysis and enhancement. The product of this synergistic effort is a powerful new analytical tool with applications in areas ranging from biomedical diagnostics to weapons materials characterization.

DESCRIPTION

The objectives of this project were: (1) to develop a Confocal Hyperspectral Imaging Raman Microscope (CHIRM), and (2) using modern image processing technology, to devise algorithms for hyperspectral microscopic image recovery and enhancement. In both cases the objective was to develop instrumentation and software for both diagnostic and research applications, which require maximum sensitivity and minimum complexity and cost. We have completed these objectives on time and with a reduced budget.

The CHIRM instrument we have developed combines the information on molecular structure from Raman scattering (e.g. the vibrational fingerprint region) with ultimate spatial resolution (sub-micron) to produce images that reveal the location, distribution, and structure of specific molecules in the materials of interest. We have demonstrated numerous applications for such powerful and

specific diagnostic capabilities, from weapons process stream monitoring and materials characterization to biomedical research. CHIRM can be applied to the surveillance of vital structural details which determine materials properties and ultimately device performance, for example the specific changes in molecular structure that accompany aging of a high explosive formulation on the length-scale of the grain boundaries of the explosive and binder.

ERIM Int'l, Inc., applied its expertise in satellite and radar image processing, and in fluorescence microscopy, to develop algorithms and software for microscopic image acquisition, image processing and spectral enhancement. ERIM International, Inc. also collaborated in hardware development. LANL designed the hyperspectral imaging spectrograph and supplied the expertise to mate the spectrometric components to the confocal microscope. LANL also provided the experimental Raman and spectrometric expertise, and provided proof of principle and feasibility studies.

BENEFITS TO DOE

The development of CHIRM as a diagnostic technique for characterization of molecular structure with high spatial resolution has direct applications in weapons process stream monitoring and materials characterization. One specific example that we have been pursuing involves the characterization of materials aging on the molecular level, information necessary for predicting materials properties and performance. The molecular basis of materials degradation over time and as a function of environment can be addressed by examination of the change in the spatially resolved vibrational spectra. For example, exposure of HE formulations or other relevant materials to heat, chemical and/or radiolytic agents is expected to result chemical changes which are spatially inhomogeneous. Characterization of the depth, surface coverage, grain boundaries, etc. of the aged materials, in addition to a fingerprint identification of the chemical changes of aging will help to identify the critical parameters leading to materials degradation. The CHIRM technique has significant potential as a real-time, nondestructive approach for process and product certification and control.

ECONOMIC IMPACT

Biotechnology will likely be a huge growth industry for the U. S. and for its foreign competitors. According to Alan Bromley, a former science advisor to the president, sales of biotechnology products (defined to encompass genetic engineering and biomedical instrumentation products) are expected to increase to \$50 billion in the next decade, approximately the size of the semiconductor industry. Biotechnology is regarded as an important new focus for U. S. industry. Current research and development is leading to improved processes in pharmaceutical and biopolymer production and new diagnostics for human healthcare. Furthermore, advanced developments such as biomolecular computation and genetic mapping are exciting wide interest (and funding). In addition, analytical quality control requirements are increasing in the related industries. The competitive advantage of U. S. companies using biotechnology will depend on their ability to understand and improve new methods. Optical spectroscopy in general, and Raman spectroscopy in particular, are research tools of long-standing importance, and they are very amenable to studies *in vivo* or in aqueous solution. Confocal hyperspectral Raman microscopes will garner the valuable vibrational spectroscopic images from microscopic samples.

We expect the analytical capabilities of CHIRM, particularly in biomedical applications, to find wide clinical and research use. No such instrumentation exists on the market today. The market for new Raman instrumentation is fairly dependable and well known, with the sales of about \$40 million annually. With advanced, lower cost systems, we project this market to expand substantially. In particular, CHIRM technology will likely expand Raman applications significantly, including biomedical imaging, characterization of semiconductor integrated circuits,

polymers, and high-tech structural materials. Furthermore, we project that nearly half of new spectrometers would be used with microprobes if price and performance were improved.

PROJECT STATUS

The project is complete and the agreement with ERIM International, Inc. has been closed. We are continuing to use the technology on a funded project to determine the molecular basis of weapons polymer aging processes.

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ERIM International, Inc., is a high-technology organization that performs research and related services for government and private sponsors. Practical applications fall into three areas; defense, environmental assessment, and economic development. Formerly known as Willow Run Laboratories and the Environmental Research Institute of Michigan (ERIM), ERIM International, Inc. was originally part of the University of Michigan. Prior to World War II they were the developers of airborne radar. Since the war they have been at the forefront of radar imaging developments (e.g. SLAR and SAR) and have become heavily involved in satellite imaging for defense and other classified as well as environmental applications. ERIM International, Inc. has for some time been independent of the University of Michigan and has approximately 600 employees.

PROJECT EXAMPLES

This project has produced a working prototype of a confocal, hyperspectral imaging Raman microscope. Demonstrations of the microscope are possible with adequate advance notice. In addition, the technology has been reported in the peer reviewed literature, and reprints are available upon request.