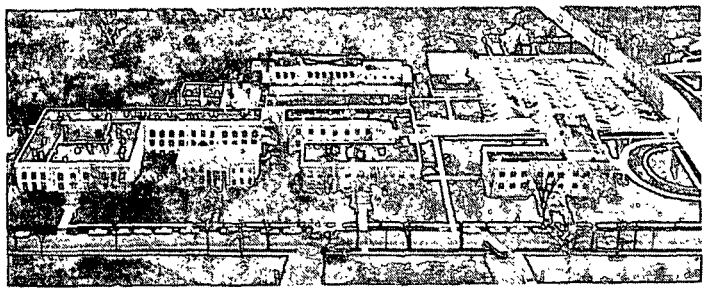
DOE DE-FG-05-85ER75212



THE INSTITUTE OF PAPER CHEMISTRY, APPLETON, WISCONSIN

TIME RESOLVED RAMAN MICROPROBE SYSTEM

Grant No. DE-FG-05-85ER75212

Progress Report One

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EXECUTIVE SUMMARY

The objective of the program is to provide an instrument system capable of generating a new class of information about molecular structure and organization in the structural tissues of plants. The system is intended for time-resolved Raman microspectroscopic studies of plant tissues.

The conception of the system described in the proposal was based on state-of-the-art instrumentation available at the time. It allowed for the possibility of two alternative types of laser excitation, continuous or pulsed, and included a monochromator equipped with a gatable detector, and entry optics for coupling either to a microscope or to a chamber for macroscopic samples. Thus, the intent was to allow experiments with two kinds of excitation, and with two classes of samples.

After award of the grant, and upon commitment of the Institute to provide funding beyond that initially proposed in the cost sharing program, an alternative concept was adopted. This was based on assembly of two relatively independent though more simple subsystems, which could be coupled to each other, but which could also be used independently and simultaneously. Such a design allows more efficient execution of the programs proposed, and was the one implemented and now operational.

Though some difficulties were encountered due to failure of some of the component subsystems purchased, these have now been overcome, and we continue to improve the system in an evolutionary manner. Some representative spectra acquired with the different subsystems are included in the report.

Future efforts will focus on the programs outlined in the proposal, and on further enhancements of the capabilities of the system.

INTRODUCTION

The objective of the program funded by the DOE URIP grant is to provide an instrument system capable of generating a new class of information about molecular structure and organization in the structural tissues of plants. The system was intended to enable us to carry out Raman microspectroscopic measurements on plant tissues under investigation in our own programs, as well as on materials generated in a number of other investigations underway in the laboratories of colleagues active in related fields of plant biology.

The conception of the system presented in the proposal was based on the possibility of two alternative types of laser excitation, continuous or pulsed. The monochromator was to be equipped with a gatable detector, and entry optics for coupling either to a microscope or to a chamber for macroscopic samples. Thus the intent was to allow experiments with two kinds of excitation, and with two classes of samples.

After award of the grant, and upon commitment of the Institute to provide funding beyond that initially proposed in the cost sharing program, it seemed that an alternate concept, based on two relatively independent though more simple subsystems, would be more effective for execution of the proposed programs. This is the plan which has been followed and which has been effectively implemented.

In the following sections the basic concept for the two related subsystems is outlined first. Each of the subsystems is then described, and representative spectra from each are presented. Some of the difficulties which had to be surmounted in order to achieve successful operation are outlined. Finally, the plans for future work are summarized.

SYSTEM DESIGN

The concept for the system initially envisioned and described in the proposal was based on designs of the state-of-the-art commercial systems available at the time. Each of these systems was built around a single monochromator equipped with an optical system allowing interfacing alternatively with either a microprobe or a macrochamber designed for investigating macroscopic samples.

Immediately upon confirmation of the award of the grant we visited a number of the manufacturers of commercial systems to assess the merits of their systems and to solicit firm quotations. We discovered that all of the manufacturers attached a substantial cost component to the possibility of interfacing the monochromator alternatively with the microprobe or the macrochamber. It was clear also that the costs attributed to the detector systems were substantially above those quoted by the detector manufacturers. As a result we decided to explore the economies which could be achieved by purchasing the subsystems directly from their manufacturers and assembling them ourselves.

As the additional flexibility allowed by assembly of our own systems was integrated into our planning, we considered the possibility of acquiring two separate monochromators, one to be dedicated to the microprobe, the other to studies of macroscopic samples. This would also require the acquisition of two detector systems. The primary attraction of this approach was that we would then have two subsystems that were operable independently of each other, and far greater efficiency would be achieved; while one subsystem was being utilized with continuous laser excitation, the other could be used with the pulsed laser source. Idle time for both programs and equipment would be reduced to a minimum.

Because of the many attractions of our alternative approach the Institute committed itself to providing the additional funding required beyond that initially envisioned in the proposal. The two subsystems have now been assembled and are operational.

In the present configuration the pulsed laser and the gatable detector are coupled to the monochromator dedicated to macroscopic samples. The continuous laser and the nongatable detector are coupled to the monochromator dedicated to the microprobe. The subsystems are so arranged relative to each other that it would be easy to switch the exciting lasers between the two subsystems.

MICROPROBE SYSTEM

This subsystem consists of a triple monochromator with a microscope attached to it and coupled with its entrance slit, and a diode array detector mounted in the plane of the exit slit. The triple monochromator is a Spex model 1877A, consisting of a filter stage, including two short focal length monochromators with opposed dispersion, and a third dispersive stage of longer focal length. The first two stages can be equipped with either 300 or 600 grooves/mm gratings, while the third stage has interchangeable 600, 1200, and 1800 grooves/mm. The monochromator can be adjusted such that one can distribute a spectral interval as narrow as 150 cm^{-1} over the full width of the detector, at one extreme, for maximum spectral resolution, and, at the other extreme, it can be set such that a broad interval of the order of 2000 cm^{-1} can be monitored by the same diode array, for maximum spectral coverage, albeit at considerable sacrifice in spectral resolution.

The microscope system is the Spex model 1482 Micramate, built around a Zeis WL research grade microscope stand. It allows entry of the laser beam

above the nosepiece, for coupling into the optics of the microscope with a beam splitter. The microscope has also been modified to interface it with the spectrometer. The coupling optics in essence form an image of the object under study which is illuminated by the focused laser beam, at the entrance slit of the spectrometer.

The detector is a Tracor Northern model TN-6122A equipped with a model TN-6500/BDCBA data acquisition system. It consists of an intensified diode array with approximately 1000 active diodes. It is approximately 2.5 cm wide, and can be adjusted so that it is precisely positioned in the plane of the exit slit of the spectrometer. Of course in the present instance the spectrometer does not have an exit slit. Rather the spectrometer system forms an image of the entrance slit in the plane of the diode array, and the different elements of the array then detect the images formed at different frequencies. Thus it is possible to acquire the spectra at 1000 different frequency increments simultaneously.

The laser currently used with the microprobe system is a Coherent continuous argon ion laser that was available at the Institute and in use in another program. It is rated at 3 watts and is model no. 90 in the Innova series made by Coherent.

In illustration of the capabilities of the new spectrometer system we attach copies of some spectra which were acquired quite recently. Figure 1 includes four spectra acquired from a point on the surface of a cell of ramie. In this study we were seeking to find if any changes in the organization of cellulose occur when a never dried sample of ramie is dried in air. The spectra on the left hand side of the figure were recorded for the never dried tissue

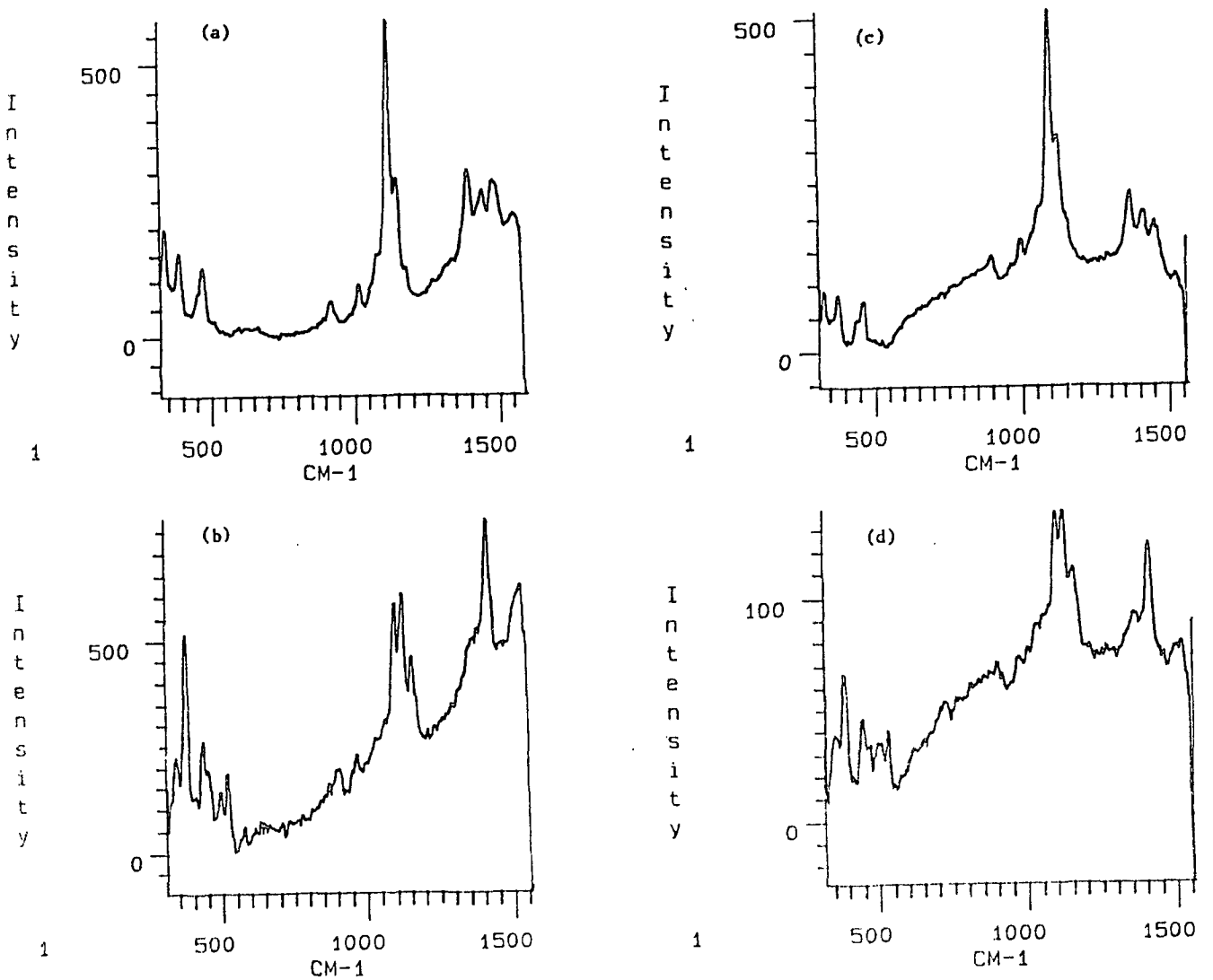

Figure 1. Spectra of the surface of a cell of ramie.

a. Never dried cell immersed in water with laser polarization parallel to cell axis.
b. Never dried cell immersed in water with laser polarization perpendicular to cell axis.
c. Air dried cell with laser polarization parallel to cell axis.
d. Air dried cell with laser polarization perpendicular to cell axis.

immersed in water; the upper spectrum is recorded with the polarization of the laser parallel to the cell axis, and the lower one is recorded with the polarization of the laser perpendicular to the axis of the cell. The spectra on the right hand side of the figure were recorded in a manner analogous to those on the left side, but after the cell was allowed to dry in air at room temperature. While there are differences in the underlying background luminescence, the main features in the spectra are quite similar.

The major point to be made in connection with Figure 1 is that each of the spectra was acquired in 8 to 10 minutes. With our earlier spectrometer, which operates with single channel detection, spectra of comparable signal to noise ratio would have required 4 to 6 hours each.

MACRO SYSTEM WITH ULTRAFAST LASER EXCITATION

This subsystem also includes a triple monochromator equipped with a diode array detector, but in this instance the monochromator is coupled to an optical platform holding the components necessary for investigating macroscopic samples, and the detector is gatable. That is, the intensifier on the diode array can be switched on and off electronically in synchrony with the pulses of the exciting laser.

The triple monochromator is a Spex model 1877B, similar to the one described in detail above, except that it is equipped with a scanning drive permitting its use as a scanning spectrometer if such use becomes desirable. Thus it also has a filter stage, consisting of two short focal length monochromators with opposed dispersion, and a third dispersive stage of longer focal length, and can be adjusted so that different spectral intervals cover the full width of the detector.

Here again the detector is a Tracor Northern system. It consists of the model TN-6122A detector head equipped with a model TN-6500/BDCBA data acquisition system. As in the detector attached to the microprobe, it has approximately 1000 active diodes, is approximately 2.5 cm wide, and is adjustable to allow precise positioning in the plane of the exit slit of the spectrometer. As noted earlier, in the present instances the spectrometers do not have exit slits. Rather they form images of the entrance slits in the planes where the exit slits would be mounted in traditional scanning spectrometers.

The pulsed laser currently used with this system is the Coherent Ultrafast 702-1 dual jet dye laser pumped by a mode locked and frequency doubled Quantronix model 416 YAG laser. The YAG laser operated in the mode we use produces 100 picosecond pulses at 532 nanometers. These emerge from the dye laser approximately 1 picosecond wide when both jets are used, or 6 to 8 picoseconds wide when only one jet is used.

In illustration of the capabilities of this subsystem, we attach in Figure 2 some spectra which were acquired quite recently. They illustrate the possibility of discriminating against fluorescence. One of the spectra was recorded from a sample of ground wood without gating of the detector, the other spectrum was recorded with the detector gated to suppress fluorescence. It is clear that the level of background fluorescence has been reduced. The difference is even more noteworthy when one considers that the spectrum recorded without gating was recorded approximately one hour after the gated spectrum. Such extended exposure to the laser beam usually results in a quenching of the fluorescence. Thus the capacity of the system to discriminate against fluorescence is in fact greater than suggested by Figure 2.

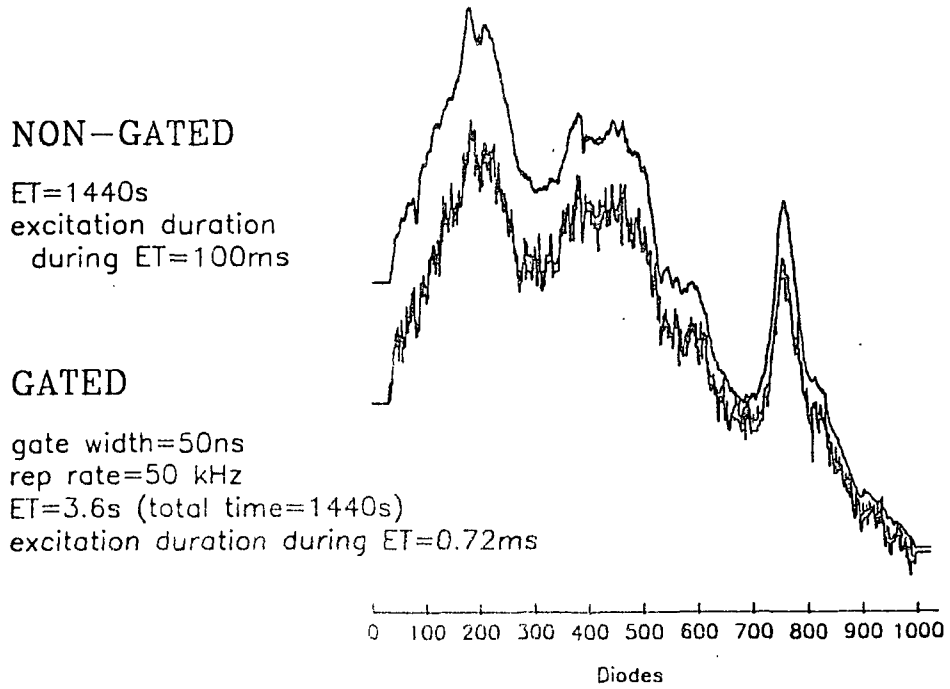

Figure 2. Comparison of gated and nongated spectra of groundwood handsheets immersed in water. The nongated spectra were recorded approximately one hour after the gated spectra, so that the discrimination against fluorescence is in fact greater than suggested by the comparison above.

Furthermore, these are among the earliest spectra recorded in this effort, so that we expect to achieve far more significant discrimination against fluorescence as we develop greater experience in the operating characteristics of the experimental system.

Another point to be noted in connection with Figure 2 is that the spectra were acquired with extremely short exposures. An even more dramatic illustration is the spectrum of glucose shown in Figure 3, which was acquired with an effective exposure time of 1.8 microseconds. This is to be contrasted with acquisition time of the order of 20 minutes for a spectrum of comparable signal to noise quality acquired in a scanning spectrometer. In this respect the performance of the system is beyond our earlier expectations, as we had expected the spectra acquired to be related to the average power of the laser in a manner similar to that of a continuous laser. Instead we find spectra which are considerably enhanced relative to those that would be acquired with excitation by a continuous laser of equal power.

PROBLEMS

Completion of the systems has been delayed relative to the originally anticipated schedule because of a number of unanticipated problems encountered as the different components were received and installed. The problems encountered with the systems were in two different categories. The first were related to a disproportionate number of defective components received from the suppliers, as well as failures of some components very soon after installation. The second were related to mechanical instabilities of the microscope coupling to the spectrometer in the microprobe. These problems have now been overcome, though we continue to improve the systems in an evolutionary manner. As we do so, we

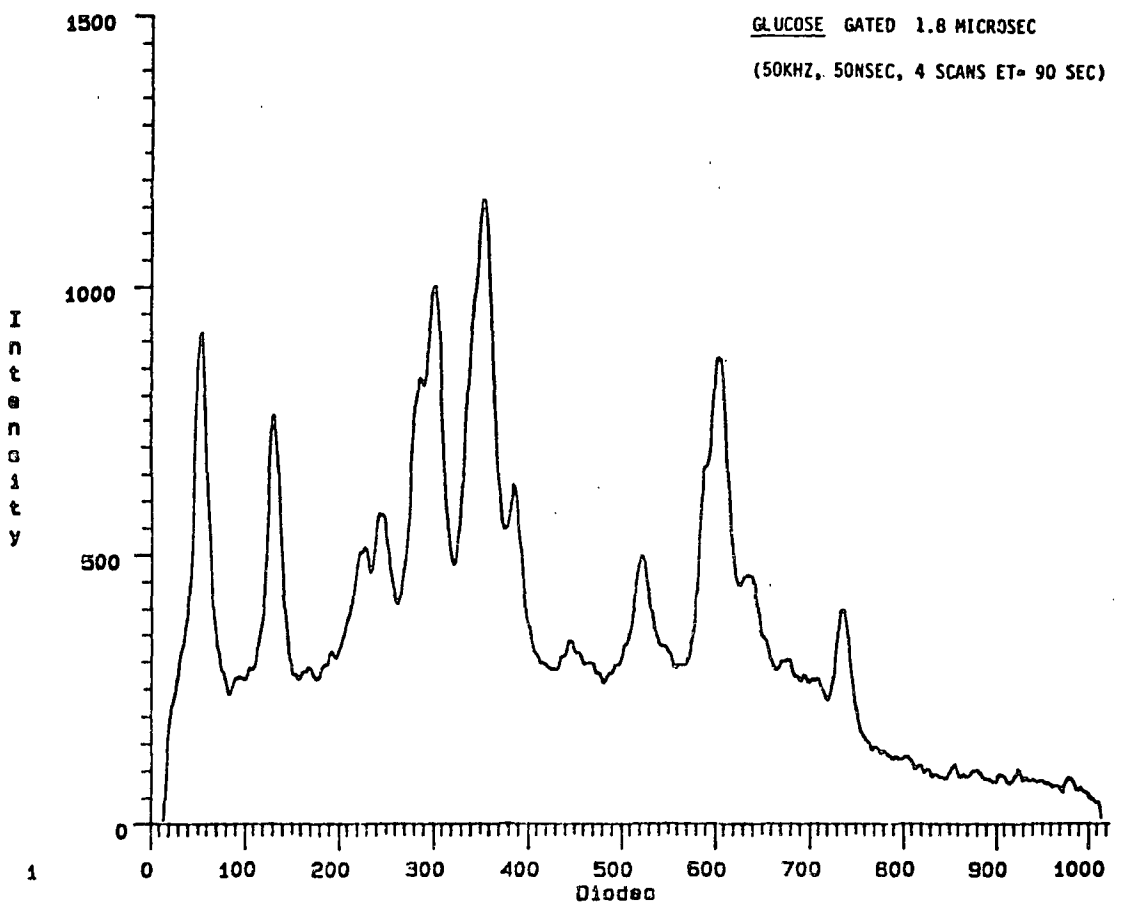

Figure 3. Raman spectrum of glucose recorded in the gated mode to test the efficiency of the spectrometer system.

will prepare technical notes to be published in instrumentation research journals, and we will include these in future technical reports in relation to the present grant.

FUTURE PROGRAMS

The primary effort in the coming months will be devoted to the programs of research currently in progress with the support of DOE grant No. DE-FG02-84ER13189, entitled "Raman Microprobe Investigation of Molecular Structure and Organization in the Native State of Woody Tissue." This is a program directed at exploring the organization of both the polysaccharides and the lignin in the cell walls of woody tissue. As indicated in the instrument proposal, when the procedures have been well established, we expect to expand this effort to include studies of other classes of plant tissue which are of interest. Among these will be tissues generated in the research programs of colleagues both at the Institute and elsewhere.

In addition to the programs outlined in the proposal, we expect to continue to enhance the capabilities of the instrument system by evolutionary improvements in the methodology for its use. Two such enhancements are currently under development. The first involves interfacing the microprobe to an image analysis system to be used in mapping the cell wall structure and correlating molecular organization with position in the cell wall. The microprobe imaging system has been equipped with a binocular phototube equipped with a color video camera. This will be interfaced with an 80386 microprocessor based microcomputer system containing an image acquisition board, and holding image analysis software. This enhancement is expected to be in place by the end of summer 1988.

The second enhancement is more a procedure development program, and is directed at use of ultrafast laser excitation with the microprobe. The key to this is developing the capability of measuring the spectra with a power that is low enough to avoid damage to the microscope objectives. Although the average power of the pulsed laser is similar to the levels currently used in continuous excitation, the peak power levels in individual pulses is considerably higher, so that the procedures have to be approached with caution, particularly when the more expensive high quality imaging objectives are used.

As we progress in our enhancements of the instrument, and as we develop new procedures, we expect to publish accounts of our progress, and these will be appended to future technical reports related to the present grant.