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Resonance Raman Excitation
Study of Monomeric and Dimeric
Chlorophyll *a* in Solution

by

Danièle L. Thibodeau, B.Sc., M.Sc.

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of the requirements of
Doctor of Philosophy

Department of Chemistry

Carleton University

Ottawa, Ontario

June 30, 1988

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The undersigned hereby recommend to
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acceptance of the thesis,

Resonance Raman Excitation Study of
Monomeric and Dimeric Chlorophyll *a* in Solution

submitted by

Danièle L. Thibodeau, B.Sc., M.Sc.

in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

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ABSTRACT

Chlorophyll *a* (Chl *a*) was studied in two different solvent systems. In collidine, absorption and fluorescence spectroscopy indicated that Chl *a* was present as a monomer, while the spectroscopic techniques showed that Chl *a* was primarily in the form of an aggregate. Derivative spectroscopy was also used, and proved to be a rapid test of the state of aggregation of Chl *a* in solution.

High resolution excitation profiles for several bands of Chl *a* were measured over a large portion of the Soret absorption region (403-451 nm). Two strong Raman bands assigned to the macrocycle skeletal modes were monitored in both solvent systems. They correspond to the C-N (1189 cm^{-1}) and C_b-C_b (1555-1560 cm^{-1}) stretching modes.

In hexane, where Chl *a* is aggregated, 1655 cm^{-1} and the 1705 cm^{-1} bands were examined. They correspond to a "bound" and a "free" C₉=O respectively. These carbonyl bands have been chosen because they are involved in the binding of aggregate and/or dimeric forms of Chl *a*. The Raman data obtained from this work confirmed the presence of a dimer in which one molecule is bound to the other through a C₉=O bond. This result is consistent with a T-shaped Chl *a* dimer, although the angle between the two molecules cannot be established from the Raman data.

Results from this work have confirmed that the excitation profiles of these modes in both solutions are governed by the Franck-Condon overlap factors. This conclusion is supported by the following observations: (i) the excitation profiles followed the absorption spectra, and (ii) the resonance Raman intensities were enhanced in the region of the first vibronic band of the Soret absorption band.

For the monomeric solution, the excitation profile enhancement of the two skeletal modes of vibration agreed with a theoretical excitation profile based on a transform method which assumes Albrecht's A term scattering.

Some initial work has been done to calculate the resonance Raman excitation profile (REP) by a transform method based on the absorption spectrum of Chl *a* in collidine. Theoretical REPs were obtained and compared for both the Soret (blue) and the Q_y (red) regions. Both REPs were different, reflecting the different properties of the electronic excited states that they represent.

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Danièle Thibodeau
June 1988

TABLE OF CONTENTS

ACCEPTANCE SHEET	ii
ABSTRACT	iii
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	viii
LIST OF FIGURES	xi
LIST OF TABLES	xiii
QUOTE	xiv
DEDICATION	xv

1. INTRODUCTION	1
2. STRUCTURE AND SELECTED PROPERTIES OF CHL <i>a</i>	6
2.1 MOLECULAR STRUCTURE OF CHL <i>a</i>	6
2.2 COORDINATION OF THE MAGNESIUM ATOM	8
2.2.1 Solvent effects	9
2.3 ABSORPTION SPECTROSCOPY OF CHL <i>a</i>	12
2.3.1 Solvent and Concentration Effects	14
2.3.2 Advantages and Limitations of Absorption Spectroscopy	15
2.4 RAMAN SPECTROSCOPY	16
2.4.1 Recent Development in Resonance Raman of Chl <i>a</i>	17

3. THEORY	
3.1 INTRODUCTION	19
3.2 OPTICAL SPECTROSCOPY	19

3.3	ABSORPTION SPECTROSCOPY	21
3.3.1	Dimer Models	22
3.3.2	Advantages and Limitations of Absorption Spectroscopy	23
3.4	RAMAN SPECTROSCOPY	23
3.4.1	Polarizability Derivative and Vibronic Expansion	27
3.4.2	Raman Excitation Profile	29
3.4.3	Resonance Raman Scattering	31
3.5	DERIVATIVE SPECTROSCOPY	44
4.	EXPERIMENTAL SECTION	
4.1	INTRODUCTION	49
4.2	PIGMENT EXTRACTION	49
4.3	CHLOROPHYLL <i>a</i> PURIFICATION	50
4.4	CHLOROPHYLL <i>a</i> SAMPLE PREPARATION	53
4.4.1	Chl <i>a</i> in Hexane	53
4.4.2	Chl <i>a</i> in Collidine	54
4.4.3	Method to Evaluate Chl <i>a</i> Content	54
4.5	CELL DESIGN AND ASSEMBLY	56
4.5.1	Sample Cells	56
4.5.2	Flowing System	56
4.6	INSTRUMENTATION	58
4.6.1	Lasers	59
4.6.2	Sample Device	61
4.6.3	Monochromator Spectrometer	64
4.6.4	Detection System	65
4.7	DATA ACQUISITION	71
4.7.1	Hardware	71
4.7.2	Software	75
4.7.3	Software Programs	76
4.8	OTHER EXPERIMENTAL CONDITIONS	81
4.9	TECHNICAL CONSTRAINTS	81

5. RESULTS AND DISCUSSION

5.1	INTRODUCTION	84
5.2	DATA ACQUISITION	85
5.3	DERIVATIVE SPECTROSCOPY	89
5.4	RESONANCE RAMAN STUDY OF CHL <i>a</i> , IN COLLIDINE	94
5.4.1	Resonance Raman Spectra	94
5.4.2	Raman Excitation Profiles	97
5.4.3	Theoretical Calculations of Excitation Profiles	97
5.5	RESONANCE RAMAN STUDY OF CHL <i>a</i> IN HEXANE	101
6.	CONCLUSIONS	107
	REFERENCES	109
APPENDIX A.		
	Calculation to Evaluate Chl <i>a</i> Content	120
APPENDIX B.		
	Glossary of Technical Terms	121
APPENDIX C.		
	Listings of Computer Programs	123

LIST OF FIGURES

2.1	Molecular Structure and Numbering Schemes of Chl <i>a</i> .	7
2.2	Relative Position of Two Molecules of Ethyl Chlorophyllide <i>a</i> .	10
2.3	Electrostatic Effect of 1 or 2 Solvent Molecules on the Conjugation Pathway of Chl <i>a</i> .	11
3.1	IR and Raman Processes.	24
3.2	Relationship Between the Sum-Over-State, Time-Dependent and Transform Theories.	32
3.3	Energy Level Diagram of the Raman Process.	33
3.4	Intermediate Steps of the Transform Method.	43
3.5	Characteristic Profiles of Derivation of Gaussian Curves.	48
4.1	Steps for the Extraction and the Purification of Chlorophyll <i>a</i> .	55
4.2	Schematic Diagram and Dimension of the Flowing Cell.	57
4.3	Schematic Diagram of the Nanosecond Laser Raman System.	60
4.4	Position of the Optical Components.	63
4.5	Intensity of Raman Signal as a Function of High Voltage Applied on PMT for RCA 3134A and RCA 8850.	66
4.6	Signal-to-Noise Ratio for RCA 3134A and RCA 8850 as Function of High Voltage.	67
4.7	Raman Signal Intensity as Function of Laser Flux for RCA 8850.	68
4.8	Configuration of RS232 Cable.	73

5.1	Improvement on S/N Ratio as a Result of Averaging Consecutive Scans.	87
5.2	Improvement on S/N Ratio as a Result of Averaging Pulses.	88
5.3	Absorption and 2 nd Derivative Spectra of Chl <i>a</i> as Monomer and Dimer.	92
5.4	Characteristic Profiles of Two Chl <i>a</i> Bands in Solution in Hexane.	93
5.5	Resonance Raman Spectrum of Chl <i>a</i> in Collidine.	95
5.6	Raman Excitation Profiles of Chl <i>a</i> in Collidine for Two Raman Shifts.	96
5.7	Comparison of the Q _y Band in Shape and Position of First Vibronic Transition with the Soret Region.	99
5.8	Calculated Excitation Profile of the 1189 cm ⁻¹ Mode by Transform Method from the Absorption in the Soret and the Q _y Regions.	100
5.9	Resonance Excitation Profiles of 1555 cm ⁻¹ Mode.	102
5.10	Resonance Excitation Profiles of 1655 cm ⁻¹ Mode.	103
5.11	Resonance Excitation Profiles of 1705 cm ⁻¹ Mode.	104
5.12	Raman Spectra of Chl <i>a</i> in Hexane at Two Exciting Frequencies.	105

LIST OF TABLES

3.1	Notation used for Mathematical Formulae.	30
4.1	Table of Laser Dyes Pumped with N ₂ Laser.	61
4.2	Configuration Switch Settings of the SR245 Interface.	72
4.3	SR245 interface most commonly used commands.	74
4.4	Pin Configuration between Sr245 interface module and Ramanor HG2 Computer.	75
4.5	Raven Computer Features.	76
4.6	Lists of Computer Programs.	80
5.1	Absorption and 2 nd Derivative Peak Positions of Chl <i>a</i> Solution in Hexane.	91

*Machines exist, let us then exploit them
to create beauty — a modern beauty, while
we are about it. For we live in the
twentieth century.*

Aldous Huxley

Cette thèse est dédiée
A mes parents, Gilles et Yvette
A mes frères et soeur, Claude, Michel et
Guylaine.

1. INTRODUCTION

Nature's provision of cheap efficient "solar cells" in the form of photosynthetic organisms has launched numerous efforts during the past two centuries to understand photosynthesis. The elucidation of the complex physical, chemical and biological process goes beyond mere scientific curiosity and extends into more practical pursuits. If man could effectively reproduce or modify the mechanism present in photosynthetic bacteria or plants, he would have a means of producing cheap photochemical batteries.

The chlorophylls (Chl) are characteristic and common representations of the group of pigments in photosynthetic organisms.^{1,2} They are involved in several steps in the primary reaction of photosynthesis³: light absorption, excitation energy transfer, transduction of excitation energy into electrochemical energy⁴, and most probably in the early stages of electron transfer⁵. Because of their importance in photosynthesis, the Chls have been extensively studied in terms of their chemistry⁶, electronic structure⁷, spectroscopic properties⁸, and functional properties⁹.

It is recognized that there are various Chl species and types of self-coordination or associations which perform different functions in the organism which are collectively called the "light reactions". A recent monograph³ reviews the techniques used to elucidate the relationship between the structure and function of the photosynthetic apparatus. Perhaps the most significant breakthrough in photosynthesis

research to date has been the x-ray crystallographic work of Deisenhofer *et al.*¹⁰ which gives a full structural description of the reaction centre of the bacterium *Rhodospseudomonas virus* to about 3 Å resolution.

Resonance Raman spectroscopy has proven to be a valuable tool for studying photosynthetic pigments in their complex native protein membranes¹¹. Because it is a vibrational spectroscopy, it yields information on a molecule's ground electronic state at a submolecular level (that is, submolecular resolution, which is less than 3 Å). Because it is a resonance spectroscopy, resonance Raman spectroscopy is highly selective over other pigments or protein substitutes which would contain similar vibrational species that may distort or even mask the vibrational band of interest. The use of low-energy photons, which are those in resonance with the photosynthetic apparatus constitutes only a mild perturbation, which to first order, is not destructive.

Much of the information obtained from the resonance Raman spectra of Chls has for a long time been obtained in *in vitro* studies using other molecular spectroscopic methods^{12,13}. The Chl-Chl interaction has been studied by magnetic resonance¹⁵, electron spin resonance¹⁶, visible absorption¹⁷, circular dichroism and magnetic circular dichroism¹⁸. These studies reveal the presence of various coordinated Chl species in various degrees of association. In particular, the monomeric, dimeric, and aggregated Chls (or oligomers) are of most interest because these species provide models for actual species present in the photosynthetic apparatus. Spectroscopic evidence¹⁹ has shown that the light-harvesting complexes (LHC) are bonded to molecules other than Chls, whereas Chls in the reaction centre

(RC) show signs of interaction with themselves or other similar pigments. The Chls in the RC are believed to be assembled in dimeric species as revealed by ESR²⁰, their optical spectroscopic properties²¹, and by x-ray crystallography¹⁰.

The aim of the work in this thesis is to demonstrate the potential of nanosecond pulsed laser Raman spectroscopy to yield new information on the electronic energy levels in monomeric and dimeric forms of chlorophyll *a* (Chl *a*). The knowledge gained from the study of the monomer will lead to a better understanding of the interactions of individual units when they are assembled as a dimer.⁶ This work was also carried out to obtain some insight into the structural properties of dimeric Chl *a* through the study of the molecular binding forces. This will be accomplished by comparing the absorption spectrum with the analysis of high resolution Raman excitation profiles over an extended range of the Soret absorption region (400 – 450 nm).

Resonance Raman spectroscopy provides information about excited-state structure and dynamics²¹. The vibrational frequencies provide information about geometry and the electronic structure of the chromophore in its ground state. In addition, the intensities of the Raman bands contain information about the symmetry, equilibrium geometry, and dynamics of the resonant electronic state. This is particularly useful in large biological molecules which often show broad, diffuse absorption bands which provide little structural information. Also, in many biological chromophores such as Chl *a*, the most interesting excited states are often short-lived as a result of photochemistry (as in the Q_y band)²³ or energy transfer (as in the Soret band)²⁴. In these cases, resonance Raman spectroscopy may provide the most direct approach to study excited-state structure.

The relationship between resonance Raman intensities and excited state structure has been known since the early work of Tang and Albrecht²⁵. The evolution of resonance Raman theory has seen the traditional sum-over-states formalism, and the more recent time-dependent wavepacket description of Hizhnyakov and Tehver²⁶, and Heller²⁷, become more manageable in terms of relating observed Raman intensities directly with excited-state parameters. This is mainly the result of the recent theoretical approach of transform methods^{28,29} which represents a third class of theoretical framework which is extremely well-suited to obtain excited-state information directly from experimental data and circumvents the computational problems often encountered with large molecules in the sum-over-states picture.

The thesis is divided into six chapters which are organized in the following way:

- Chapter 2 draws attention to the background knowledge for the study of chlorophylls and the recent developments related to Resonance Raman of chlorophylls.
- Chapter 3 introduces the theoretical concepts necessary to the analysis of the data presented in this work.
- Chapter 4 gives a description of experimental procedures. It includes a section on the implementation and evaluation of an automated data acquisition system for the recording of nanosecond pulsed laser Raman spectra.

Chapter 5 presents the results of the experimental observations and their theoretical calculations are discussed.

Chapter 6 reports a summary of the results and conclusions of this work.

2. STRUCTURE AND SELECTED PROPERTIES OF CHL *a*

2.1 MOLECULAR STRUCTURE OF Chl *a*

In the 1930's Fischer *et al.*³⁰ proposed the structure of chlorophyll which was confirmed 30 years later by its synthesis by Woodward *et al.*³¹. Other refinements, mostly concerning the configuration at the chiral centres followed rapidly.

Even though IUPAC have proposed systematic names for the coordination complexes of tetrapyrroles³² the Fischer nomenclature and numbering system have imposed themselves in the literature. Fig. 2.1 represents schematically the molecular formula of Chl *a* pigments using both types of nomenclature and numbering systems.

Chlorophylls are characterized by:

- i) a central Mg (II) ion;
- ii) chlorin ring reduction of a pyrrole ring in the tetrapyrrole macrocycle;
- iii) a phytol chain;
- iv) methyl, ethyl, vinyl and propanoic acid as peripheral substituents;
- v) a pentanone ring.

Figure 2.1

Molecular structure and numbering schemes of Chl *a*

a) Fischer b) IUPAC

The loss of a double bond in the macrocycle sensitizes the molecule to the red end of the visible part of the electromagnetic spectrum. The long phytol chain gives to chlorophyll its lipophilic properties. The peripheral groups alter its electronic characteristics. All these properties, without doubt, are the result of a long-term fine-tuning process. But it is the Mg ion together with the carbonyl group on ring V that gives Chl *a* its unique electron acceptor-donor property.

2.2 COORDINATION OF THE MAGNESIUM ATOM

The chlorin ring of Chl *a* provides four-fold coordination to the Mg atom. In this configuration, the metal ion remains an electron acceptor. The unsaturation can be relieved by accepting up to two additional ligands. These ligands can be supplied by solvent molecules or nucleophilic groups of adjacent Chl molecules. The most likely candidates for these types of interactions are the following chlorophyll functional groups: vinyl, carbonyl and aldehyde.³⁴

It was determined by IR, ¹H and ¹³C NMR spectroscopy that the carbonyl in position 9 was linked to the Mg atom in aggregates.^{12,35} The two molecules in the dimer should be non-equivalent but appear indistinguishable in NMR spectroscopy.¹² This suggests that there is a dynamic exchange of the donor-acceptor role that is faster than 1 ms. At first, it was thought that the two molecules were perpendicular to each other to improve the carbonyl-coordination and to reduce the electronic repulsion from the porphyrin ring.³⁶ But this model did not conform to the circular dichroism experimental data.³⁷ One of the goals of the work done for this thesis was to determine the structure of the dimer based on the assumption of a T-shaped model

dimer even though the angle between the two molecules is probably different from 90° . A simple calculation of electrostatic forces revealed that the two molecules lie at an angle from each other. Figure 2.2 demonstrates the result of this calculation performed with AlchemyTM, a software program run on an IBM PC clone.

2.2.1 Solvents Effects

Chlorophylls, owing to their various functional groups, can be dissolved in a wide range of solvents. Dissolution in polar solvents occurs through the binding of the Mg ion on either side of the macrocycle.³⁸ To minimize the electrostatic repulsion, the solvent molecules are positioned perpendicularly to the macrocycle. It makes a difference on the spectroscopic properties of Chl whether the macrocycle is attached to one or two additional ligands. Tasumi³⁹ explained that when one ligand is attached to the Mg ion, the Mg is displaced outside the plane of the ring. But when two equivalent molecules are ligated to the central atom, their electrostatic effects cancel, and the Mg atom remains in the plane of the ring (see Figure 2.3). Therefore, the conjugation path is different for these two configurations and it is reflected by small but distinct spectroscopic features.⁴⁰

Figure 2.2

Relative position calculated from the electrostatic forces of two molecules of ethyl chlorophyllide *a* (an ethyl substituent replaces the phetyl chain).

Figure 2.3

Representation of the effects of 1 or 2 solvent molecule(s) attached to the central Mg atom of Chl *a*.

2.3 ABSORPTION SPECTROSCOPY OF Chl *a*

Application of a model for chlorophyll has been successfully based on the molecular orbital theory of porphyrins.^{33,41} The model is built starting from the basic structure of a porphyrin ring. This 16-atom ring is made of the combination of 4 pyrrole rings that share 18 π electrons. The basic ring has a four-fold symmetry. The dipole associated with the π electronic transitions of large planar porphyrin lie in the plane of the molecule.^{8,41} The main absorption bands are characteristic of a $\pi \rightarrow \pi^*$ transition by their intensity. The bands are located in two regions of the visible spectrum, with one very strong band in the 350-450 nm range and another one (usually less intense) in the 600-700 nm region.

The porphyrin-type optical spectra can be further perturbed by various chemical modifications to the basic structure to model. The reduction of a double bond or conjugation with substituents outside the ring ($-\text{C}=\text{O}$ or $-\text{C}=\text{C}-\text{C}$) are both likely to affect the electronic system by changing the conjugation path of the electrons. The main change from the basic porphyrin ring is a reduction of a pyrrole ring, which has the most profound effect on the conjugation pathway and its symmetry (D_{4h} to D_{2h}).^{41,43} The conjugated double bond system of chlorophyll causes absorption bands to be associated with a definite transition direction in the molecule. Moreover, energy levels and intensities are less affected by configuration interaction and by the presence of substituents with conjugated bonds. The subsequent change from the free base to a metallic complex is less dramatic since the ring reduction already has lifted the degeneracy of the square ring.

The two-fold symmetry of this compound reflects itself in a different absorption spectrum. The B (blue) and Q (quasi-allowed) bands each split into two components: B_x , B_y , Q_x , and Q_y which are related to the orientation of the molecule (see Figure 2.1)^{44,45}. Also their oscillator strengths become unequal.⁴⁴ The lowest transition (Q_y) is more strongly allowed relative to porphyrins Q bands. The other transition (Q_x) is almost parity forbidden and is relatively weak.⁴⁴ The differences in the absorption spectrum of metallochlorins and metalloporphyrins have been attributed to their respective symmetries. The electronic properties of metallochlorins promote more efficient interchromophore coupling in dimeric and larger aggregates than would be possible with the metalloporphyrins.⁴⁵

Model compounds of increasing complexity have been examined with substituents to progressively match the structure of chlorophyll. The model compounds have been studied by spectroscopic methods as varied as those used for chlorophylls. The aim of these investigations was to study the effect of the reduction of symmetry and to better understand the effect of the substituents on the modification of the conjugation system.

Another characteristic of chlorophyll is an extra isocyclic pentanone (ring V in Figure 2.1). The $C_9=O$ functional group is known to coordinate with the Mg atom of a neighbouring chlorophyll molecule to form dimers or larger aggregates.⁴⁶ At this point, the symmetry is reduced to C_1 , and the carbonyl group is a part of the closed circuit of conjugated bonds (i.e. conjugation pathway).⁴⁷

2.3.1 Solvent and Concentration Effects

Under ideal conditions, spectroscopic characteristics would be obtained from isolated molecules to reflect the properties of a single molecule. This would suppose no interaction with the molecules themselves and/or the use of an ideal solvent that would separate the molecule without changing its intrinsic properties. In other words, the goal is to obtain the properties of the molecule in the gas phase. Unfortunately, large organic molecules are notorious for decomposing well before vaporization temperatures can be reached.⁴⁸ Therefore, chlorophyll molecules studied have been diluted in various matrices or solvents that are less than ideal under the conditions mentioned. They are responsible for changes in the energy levels and the bandwidth.¹³

For a $\pi \rightarrow \pi^*$ transition, the strength of the interaction increases with the polarity of the solvent.⁴⁹ Changes in solvent polarity may result in changes in intensity, band shape and wavelength of absorption. Yet, Chl *a* in various solvents have practically the same spectral characteristics. The change in the absorption coefficient varies by only 10% over a wide range of solvents^{48,50} and represents the same excited state dynamics. The band shapes remain the same except for cases where aggregation occurs.⁵⁰ In this case, the bandwidth increases and a red-shifted shoulder appears in the Q_y band.⁴⁶ The wavelength shift is the most visible change that occurs as a consequence of the interaction with solvent molecules on either side of the porphyrin plane.⁴⁸ The interaction of the solvent with the molecule lowers the average energy of each energy level and therefore has an effect on the energy difference between electronic states. Studies have been conducted to determine the energy level in the gas phase by extrapolation from a series of measurements in solvents of various polarity (i.e. 413.9 nm, 643.9 nm⁴⁸ and 412.9 nm and 643.4 nm⁵⁰). Hexane solvates

the phytol chain and does not affect the macrocycle (main chromophore), this results in more blue shifted bands than polar solvents; hexane, however, causes aggregation.

Chlorophyll is a very ~~large~~ ^{poor} absorber of light and most studies have been done at concentrations much lower than those found in the plant.⁴⁶ Nevertheless, the resemblance between spectra of photosynthetically active preparations and of a concentrated solution of chlorophyll a in hexane in terms of band position, half bandwidth and relative areas, is striking in Photosystem I of the photosynthetic process. For this reason, hexane has been used for the studies presented in this thesis. Moreover, in the concentration range under investigation, the solution is composed mainly of dimers, albeit in equilibrium with other species.^{51,52} Collidine (2,4,6-trimethyl pyridine) was chosen to provide a monomeric solution of Chl a that would be found only in a penta-coordinated form. This is the result of steric hindrance caused by the three methyl groups.⁴⁰

2.3.2 Advantages and Limitations of Absorption Spectroscopy

The absorption spectrum of Chl a has been the subject of several studies to elucidate the photophysical processes involved during photosynthesis. Even though most of the photochemistry *in vivo* occurs in the lower energy levels (Q_y),⁵³ the higher levels have aroused the curiosity of many.⁵⁴ The major findings were:

- i) two energy levels are degenerate and form the strong band (Soret), and
- ii) other energy levels in that region of the spectrum are close to the vibronic of this Soret absorption band.

2.4 Raman Spectroscopy

Laser spectroscopic methods have established themselves in biochemical investigations because of their sensitivity to structural parameters, their ability to probe selectively, and their short pulse capabilities. Resonance Raman spectroscopy is a technique which benefits from the features offered by laser light.

Vibrational studies of Chl obtained by Raman spectroscopy yield information on ground electronic state of molecules with a sub-molecular resolution.⁵⁴ Owing to the purity of the laser line, the resonance method can excite chromophores selectively. Since Raman signals follow the temporal profile of the exciting pulse, it is possible to use the discrimination power of the detection system to segregate them from longer-lived effects.

The importance of resonance Raman spectroscopy of biochemical materials lies in the fact that the spectroscopic data obtained from isolated model compounds will more closely resemble those of the native matrix than any other spectroscopic technique. Resonance Raman spectroscopy is one of the few techniques where results obtained in *in vitro* experiments can be related directly to the *in vivo* observations.⁶

In the resonance Raman effect, only the chromophore absorbing the exciting photons will give the characteristic scattered photons, thus, the background effect is therefore greatly reduced. Owing to its selectivity resonance Raman spectroscopy can also accomodate molecules found at low concentrations in a complex matrix.^{54,55}

There are good reasons to carry out *in vitro* studies of biochemical processes. First, it is a convenient way of gaining control over the composition and the contribution of each constituent of the sample. Second, elements of difficulty such as other molecules or a more rigid environment can be introduced one at a time.

Resonance Raman spectroscopy has been a useful complement to more traditional probes of biochemical functions and structures such as absorption (IR and vis), magnetic resonance and x-ray diffraction. For instance, resonance Raman spectroscopy of *in vitro* species is used to deduce structure, self-aggregation, and coordination.⁴⁸

The first Raman spectra of Chl *a* were obtained in 1972 by Lutz⁵⁶. Since then, specific applications of this technique have been used for problems related to photosynthetic systems. In Soret resonance, about 55 modes are active in the 50-1750 cm^{-1} region.

2.4.1 Recent Development in Resonance Raman of Chl *a*

Recent resonance Raman studies on chlorophylls may be classified into two categories: *in vivo* where the chlorophylls are present in solution in its native protein environment and *in vitro* where they are present in solution. The former was extensively reviewed by Lutz^{6,11}, who points out the thrust of such research is the interest in determining the local environments, molecular interactions and conformations of the chlorophylls within their functional sites of the membrane.

Because of their obvious biological importance, metallochlorins and chlorophylls have also been under increased scrutiny in *in vitro* studies. The resonance

Raman spectra of metallochlorins are more complex than those of metalloporphyrins^{55,56} because of reduced symmetry. The resonance Raman spectra of chlorophylls are more complex still, owing to the presence of the isocyclic ring (ring V). Despite this complexity, progress has been made towards identifying Raman bands of metallochlorins from those of the metalloporphyrins based on their frequencies and intensity enhancement.^{45,57}

From these data, basic assignments of resonance Raman bands of chlorophyll have been suggested.^{11,58} Most of these assignments are primarily based on the comparison of the spectra with those of the metalloporphyrins⁵⁹, especially Mg octaethyl porphyrin⁵⁴. A recent study by Boldt *et al.*⁶⁰ clearly illustrates the effect of specific structural changes of basic Ni(II) metallochlorin in the resonance Raman spectra. The presence of Ni ion in the chlorin ring produces a non-fluorescent model compound which makes possible the study of resonance Raman spectroscopy of the Q_y band, which is otherwise inaccessible to the Mg analog (as in the case of Chl *a*). The same study was carried out for Cu(II) pheophytinate.⁶¹ Other studies of the metal-substituted Chl *a* analogs have been extensively investigated by Tasumi's group.⁶²

Koningstein's group⁶³ has used a 3 ns pulsed laser to excite resonance Raman spectra of Chl *a* in hexane solution. Their Raman apparatus provided more detailed resonance Raman excitation profiles to be measured in the Soret region. The work of this thesis is an extension of the study of Chl *a* in solution using a 3 ns pulsed laser.

3. THEORY

3.1 INTRODUCTION

This chapter will present the theoretical background necessary to interpret the results of this study, which encompasses the breadth and shape of both the electronic absorption bands and the Raman excitation profiles of Chl *a*.

The chapter is arranged in the following manner. First, principles derived from quantum mechanics are introduced. Many of the same principles are common to every type of spectral transition. Second, as it pertains to the electronic transitions of Chl *a*, there will be a discussion on the recent interpretation of the absorption spectrum of Chl *a* dimers. Third, the theoretical framework of Raman scattering will be outlined leading to the description of resonance Raman spectroscopy. This discussion will provide the background needed to predict the relative Raman intensities and therefore the shape of the Raman excitation profiles. In the last section, derivative spectroscopy will be described as a technique used to reveal small or convolved features of the absorption spectra of Chl *a*.

3.2 OPTICAL SPECTROSCOPY

Optical spectroscopy encompasses the study of the interaction of a molecule with light. The electric charge of a molecule is altered by the presence of an

oscillating electric field carried by light which induces a transition between two states. The energy of a transition is a measurable quantity. The probability of the transition is a function of the energy of the incident radiation. Information contained in a spectrum are:

- i) the wavelength which represents the energy difference between the two states involved;
- ii) the intensity of the transition which depends proportionally on the square of the light induced dipole, and
- iii) the band linewidth which depends on the properties of the upper state (e.g. lifetime, intermolecular collisions).

The frequency of light used to probe the energy level structure differentiates the various types of spectroscopy. When the frequency of light satisfies the resonance condition:

$$h\nu = E_f - E_i \quad (3.2.1)$$

with E_i and E_f as the initial and final levels of energy, respectively. The probability of an electric dipole transition between two electronic state wavefunctions ψ_i and ψ_f (with corresponding energies E_i and E_f) is proportional to the square of the transition moment M_{if} given by:

$$M_{if} = \int \psi_f^* e\hat{r} \psi_i d\tau = \langle \psi_f | e\hat{r} | \psi_i \rangle \quad (3.2.2)$$

where the operator $e\hat{r}$ is often referred to as the electric dipole operator, and $d\tau$

represents all the configuration space.⁶⁴ The same principles apply almost unchanged to all types of spectroscopy.

The symmetry properties of the two wavefunctions and the operator combine to make the integrals vanish for many of the possible molecular energy levels. Only when the integrand is totally symmetric (i.e. even) with respect to all symmetry operators does the integral have a non zero value.⁶⁴

The symmetry properties of the molecules have been used to simplify the selection rules of a transition.⁶⁴ The atomic orbitals are assumed to combine to give molecular orbitals with symmetries which reflect the molecular symmetry. Equivalent positions are considered to have the same electron density. The directions for inducing dipole moments are governed by the geometry and the symmetry of the molecule. This is especially important for electronic transitions of planar molecules such as aromatic hydrocarbons.⁶⁶

3.3 ABSORPTION SPECTROSCOPY

The property of a molecule to absorb or to dissipate a fraction of the incident radiation is measured by absorption spectroscopy. As with other types of spectroscopy, rules have been formulated to evaluate the probability of an absorption transition, often extrapolated from atomic absorption spectra. The ability to predict absorption spectra (or to assign every component) reflects the knowledge of the molecular structure (e.g. type of bonding forces and molecular interaction). Electronic transition

spectroscopy is particularly suitable to give information about the presence and nature of unsaturation, particularly double bonds and aromatic systems.

3.3.1 Dimer Models

There has been considerable interest^{66,67,68} in studying the geometrical structure of dimers of both Chl *a* and bacteriochlorophyll *a* in solution⁶⁸ and in the bacterial reaction centre⁶⁹, since the original prediction⁶⁶ that the initial charge separation in the photosynthetic reaction was due to a chlorophyll "special pair".

Exciton model calculations including charge resonance states by Schaafsma *et al.*⁷¹ were able to predict a transition at ~ 700 nm for a (Chl *a*)₂ dimer, where the two molecules are plane parallel, with a transition moment comparable to that of Photosystem I.

Recognizing that an initial charge transfer (CT) state plays an essential role in electron transfer in many chemical and biological processes⁷², Petke and Maggiora^{73,74} have carried out *ab initio* quantum mechanical CI calculations on a series of Mg porphine-porphine heterodimers to explore the relationship of geometry and environment to the position of the CT states. They found that the CT states of these heterodimers were significantly sensitive to the geometry of these dimers and the distance between the constituent molecules. The current status of such calculations and their importance with respect to photo-induced electron transfer processes and design for bio-mimetic solar energy devices are discussed in a recent article⁷⁵.

3.3.2 Advantages and Limitations of Absorption Spectroscopy

Absorption spectroscopy does not respond strongly to features other than aromatic and conjugated substituents or to the groups coupled with them.³³ The technique is otherwise a highly sensitive method for which excellent spectrometers are available. The absorption spectra of organic molecules are often broad and featureless owing to the wealth of energy levels (*i.e.* electronic, vibrational, rotational). Obtaining significant information from these spectra becomes difficult. Consequently, other types of spectroscopy have to be used to complete and/or refine the assignment of all the electronic energy levels.

The power of resonance Raman spectroscopy for *in vivo* studies is that it can directly probe the vibrations of the chromophore without the interference of the "non-resonant" background. Therefore, information from the ground and the lower excited states are available with the resonance Raman technique only from the molecules in resonance with the excitation frequency.⁶

3.4 RAMAN SPECTROSCOPY

The structure of a molecule is defined by the chemical bonds among its constituent atoms. Each bond in a molecule is characterized by several modes of vibrations (stretching, twisting, deformation) which in turn reflect the influence of the atoms or groups of atoms (*e.g.* mass, polarity), the type of binding forces, and the local environment.

Figure 3.1

Molecular processes of infrared absorption and Raman scattering. The two quantum states $|i\rangle$ and $|f\rangle$ correspond to a given vibration in the electronic ground state $|g\rangle$ and the electronically excited state $|e\rangle$. In normal Raman spectroscopy, $|e\rangle$ is a virtual state. The vibrational wavefunction are represented by: $|i\rangle$, while $|f\rangle$ brackets are used to represent electronic wavefunctions.

The complicated motion of any molecule can be described as a superposition of several harmonic motions, the normal vibrations. In each normal mode, all the atoms in the molecule vibrate with the same frequency and all the atoms pass through their equilibrium position at the same time.⁷⁶ There are always as many different normal modes as there are vibrational degrees of freedom, that means $3n - 6$ normal modes for non-linear molecules where n is number of atoms (1 additional normal mode for linear molecules). Each mode is described by one normal mode coordinate Q .

Infrared and Raman spectroscopy are related since they monitor the same molecular vibrations, albeit by two different processes (see Figure 3.1). Infrared spectroscopy involves the absorption of a photon (first order process). The probability of absorption increases when the energy of this photon matches the energy difference between two vibrational levels ($h\nu_{if}$). The infrared (IR) spectrum is represented by a plot of the intensity of absorption as a function of energy. The intensity of a Raman spectrum is always much weaker than an infrared spectrum because it involves a scattering process via the nearly simultaneous (within 10^{-13} s) absorption and emission of a photon and is a second order process. Raman intensities are usually 10^{-6} - 10^{-7} times smaller than the intensity of the exciting radiation.⁷⁷ The scattered light contains frequencies shifted ($\nu_0 \pm \nu_{if}$) from the exciting line (ν_0). The Raman spectrum shifts in absolute frequency accordingly with changes in the exciting frequency. The Raman effect results from an exchange of energy between the incident light and a vibrational normal mode of the molecule (ν_{if}).

In theory, bands arise from the motion of the whole molecule, but in practice, structural units are sufficiently independent of the rest of the molecule to display their

characteristic group frequencies.⁷⁸ Each frequency shift can be further characterized by its relative intensity or its polarization properties and by its excitation profile. The frequency position is an indication of the bond strength which is a function of the geometry, symmetry, and structure of the molecule in the ground-state. In contrast, the intensity of these lines is a property of the available excited states (e.g. linewidth and displacement coupling). The ground state properties are measurable by IR absorption, but, the excited state characteristics can only be obtained by Raman spectroscopy.^{28,79} Transitions between vibrational levels of the electronic ground state of a molecule are much lower on the energy scale than electronic transitions. Raman scattering can be observed in the visible region since the Raman lines appear in the vicinity of the visible exciting line. Thus, in spite of the weakness of the phenomenon, it is possible to extract useful information from the Raman spectra because of the high efficiency of photodetectors in the visible region.

According to classical electromagnetic radiation theory, if an electric field E_0 induces a dipole moment μ in a molecule, light scattering will happen. Generally, since the wavelength of the light field (e.g. 500 nm) is much larger than the size of the molecule (≈ 1 nm), it is assumed that the electric field will remain constant over the molecule during the transition.^{28,80} Consequently μ_{if} , the induced dipole moment from an initial state i to a final state f , is related to E_0 by the proportionality constant α . So that

$$\mu_{if} = \alpha \cdot E_0 = \langle \psi_f | \alpha | \psi_i \rangle \cdot E_0 \quad (3.4.1)$$

where E_0 is the incident energy vector, α is the polarizability tensor and

μ_{if} is the induced dipole vector. The terms are also defined in Table 3.1 in more detail.

Accordingly, the Raman cross-sections are proportional to the square of the induced dipole moment which is related to the polarizability tensor.

$$\sigma_{\text{Raman}} = \frac{8 \pi e^4 E_s^3 E_L}{9 h^4 c^4} (\nu_0 - \nu_{if})^4 \sum_{\sigma p} |\alpha_{\sigma p}|^2 \quad (3.4.2)$$

3.4.1 Polarizability Derivative and Vibronic Expansion

There are two basic ways to calculate Raman intensities. First, the Raman process can be expressed in terms of the ground-state polarizability and its dependence on the nuclear coordinates. Second, the development of the Kramers-Heisenberg-Dirac (KHD) expression can be taken as a problem of vibronic spectroscopy, and this approach has the advantage of providing some insight into the excited state of the molecule. The two approaches are briefly described below.

In 1934, Placzek described Raman activity in terms of the derivative of the molecular polarizability tensor. In this description, Raman scattering is related somewhat intuitively to the chemical structure through a concept such as atom and bond polarizability.^{81,82,83} More recently, the semi-classical formulation was developed by Albrecht⁸⁴, Shorygin⁸⁵, Krushinskii and Shorygin⁸⁶, Savin⁸⁷ and Verlan⁸⁸. It is a formulation which links Raman intensities with the electronic properties of a molecule. The Raman process becomes equivalent to the absorption

followed by an emission of a photon. Behringer⁸⁹ treated the problem purely quantum mechanically, while Jacon⁹⁰ developed a rigorous model using quantum electronics. The theoretical description of the second order perturbation theory played an important role in the development of quantum mechanics. The two main equations for the description of the Raman effect are as follows:

Polarizability derivative $\alpha_{i \rightarrow f} = \sum_k^{3n-6} \left[\frac{\partial \alpha}{\partial Q_k} \right]_0 Q_k$ (3.4.3)

Vibronic expansion $\alpha_{i \rightarrow f} = A + B$ (3.4.4)

$$A = \sum_v \left[\frac{\langle f|v\rangle \langle v|i\rangle}{E_v - E_i - E_L - i\Gamma} + \frac{\langle f|v\rangle \langle v|i\rangle}{E_v - E_i + E_L - i\Gamma} \right] \quad (3.4.5)$$

$$B = \sum_v \left[\frac{\langle f|v\rangle \langle v|Q|i\rangle}{E_v - E_i - E_L - i\Gamma} + \frac{\langle f|Q|v\rangle \langle v|i\rangle}{E_v - E_f - E_L - i\Gamma} \right. \\ \left. + \frac{\langle f|v\rangle \langle v|Q|i\rangle}{E_v - E_i + E_L - i\Gamma} + \frac{\langle f|Q|v\rangle \langle v|i\rangle}{E_v - E_f + E_L - i\Gamma} \right] \quad (3.4.6)$$

The Herzberg-Teller development for the vibronic transition is introduced in the Kramers-Heisenberg-Dirac dispersion equation. The separation of the terms in Equation 3.4.4 arises from the development carried to the first power of the nuclear displacement. The A term is dependent on the Franck-Condon overlap factors and provides a measure to which the potential surfaces of the two states involved are alike.

The B term contributes only as a result of mixing of two electronic states.⁹¹ The main conclusions of the vibronic expansion are that the totally symmetric mode derives most of its intensity from term A when not in resonance with an electronic excited state. It is assumed that there is no nuclear displacement. The non-totally symmetric mode derives its intensity only from term B and/or other higher order expansion terms. They arise from the "forbidden" character in allowed transitions.

3.4.2 Raman Excitation Profile

The excitation profile is the dependence of the intensity of a Raman band on the excitation wavenumber and such a profile can be useful in assigning features in an absorption spectrum.⁹² In practice, it is a laborious point-by-point procedure, but worth the effort since Raman excitation profiles (REP) can provide information about electronic and vibrational structure which may not be resolved in a normal absorption spectrum.^{93,94} It is an effective way to measure the displacement coupling of the excited state and therefore to gain information about the structure of the excited state.⁹⁴ REP can be built from a theoretical evaluation of the Raman intensities as a function of the excitation wavenumbers. The synthesis of REP from theoretical models is also important because it shows the understanding of the processes (*e.g.* dephasing) involved. Since the analysis of Raman excitation profiles becomes especially relevant under resonance condition, it seems appropriate at this point to introduce some aspects of resonance Raman scattering.

 Table 3.1 Notation used for mathematical formulae.

c	velocity of light
A_{ν_0}	amplitude of transition
A_0	amplitude of incident radiation
ν_0	frequency of incident radiation
ν_{ν_0}	frequency of the scattered light
$\alpha_{\sigma\rho}$	component of the scattering tensor
$\sigma\rho$	polarizability coordinates
$ F\rangle$	complete wavefunction of the final state
$ V\rangle$	complete wavefunction of the intermediate state
$ I\rangle$	complete wavefunction of the initial state
E	energy (cm^{-1})
Γ	homogeneous linewidth (cm^{-1})
$ g\rangle$	electronic wavefunction of the ground state
$ e\rangle$	electronic wavefunction of the excited state
$ f\rangle$	wavefunction of the vibrational final state
$ v\rangle$	wavefunction of the vibrational intermediate state
$ i\rangle$	wavefunction of the vibrational initial state
n	refractive index
ϵ	extinction coefficient
σ	cross-section ($\text{\AA}^2/\text{molecule}$)
μ	induced dipole moment
Δ	displacement of the nuclear coordinate
H	Hamiltonian operator
i	imaginary number
M	electronic operator operator
Q	normal nuclear coordinate

3.4.3 Resonance Raman Scattering

Resonance Raman scattering occurs when the energy of the exciting radiation falls within an electronic absorption band. It is treated as a special case of Raman spectroscopy. One of the conditions of the Placzek theory (ν_L exciting far away from $\nu_{0,0}$) no longer holds, and resonance Raman spectroscopy must be described in terms of quantum mechanics. Fortunately, the KHD dispersion theory becomes more manageable in a resonance condition, since only vibrations of the resonant state have to be considered. The structure of the Raman spectrum will depend on the specific vibrational and rotational quantum numbers of the selected excited state in the form of the Franck-Condon overlap factors containing the matrix product of the initial and final terms. In the resonance Raman effect, certain vibrational band intensities can be enhanced up to a factor of 10^3 . The modes which gain resonance enhancement of Raman intensity are limited to the vibrations involved in the chromophore of the absorption band. Excitation in first vibronics of electronic states also results in an enhancement. The increase in intensity is expected to be lower in intensity and related to the Franck-Condon overlap factors. This effect was predicted by Koningstein⁹⁵ and developed theoretically by Albrecht⁸³.

Absorption and scattering of photons are the two possible effects of the dipole moment induced by light.⁹⁶ Theoretically, correlation arises from the optical theorem which identifies the absorption cross-section with the imaginary part of the polarization.⁹⁷ The scattering intensity, however, derives its amplitude from the real part of the complex polarization of the molecule during a molecule-photon interaction. Since they are related by the complex polarization of the index of refraction, the absorption cross-section depends on the same excited state parameters as does the Raman cross-section. Although the absorption spectrum is often too diffuse to provide

Figure 3.2

Relationship between the sum-over-state, time-dependent and transform theories to analyze resonance Raman intensities and absorption spectra.

Figure 3.3

Energy level diagram of the Raman process accompanying notation in Table 3.1.

much specific information, it can be measured much more accurately than Raman intensities. The excited state parameters used to fit the Raman intensities should always be required to fit the absorption spectrum.^{28,98}

Three main concepts have been adopted to analyze the resonance Raman intensities. The explanation of the resonance Raman spectroscopy requires a quantum mechanical approach and, accordingly, the sum-over-states, the time-dependent, and the transform theory all originate from the KHD expression. They are mathematically linked as illustrated in Figure 3.2 even though their derivations and approximations may be used in a different perspective. They provide the same excited-state parameters.^{28,82,92} The theoretical grounds for the three methods are described briefly in sections 3.4.4.A, 3.4.4.B, 3.4.4.C.

As the condition for resonance Raman spectroscopy is approached, a damping factor must be included to prevent the scattering amplitude from reaching infinity when the two terms in the energy denominator (Equations 3.4.5-3.4.6) cancel each other as ν_0 approaches ν_e . The homogeneous linewidth Γ_e of the resonant state is the usual damping factor associated with the electronic excited state e .^{28,83,99}

At this point, various approximations are made to simplify the treatment of the overlapping wavefunctions. It is assumed that a single excited state is involved in the effect and only the diagonal vibronic couplings are involved. Thereafter, the two polarization subscripts have been neglected (it assumes that $\sigma = \rho$).

A. Sum-over-States Theory: In the simplified case, the resonance Raman cross-section is calculated by the sum-over-states equation. The expected intensities are mostly determined by the magnitude of the Franck-Condon overlap amplitudes (Albrecht's A term). The derivation steps and approximations are listed below. ^{28,99}

i) Starting with the Raman cross-section:

$$\sigma_{I \rightarrow F} = K E_L (\nu_0 - \nu_{IF})^4 \sum_{\sigma\rho} |\alpha_{\sigma\rho}|^2 \quad (3.4.7)$$

where K is a constant and the polarizability tensor is defined as

$$\alpha_{\sigma\rho} = \sum_V \left[\frac{\langle F | \mu_p | V \rangle \langle V | \mu_\sigma | I \rangle}{E_V - E_I - E_L - i\Gamma} + \frac{\langle F | \mu_\sigma | V \rangle \langle V | \mu_p | I \rangle}{E_V - E_F + E_L - i\Gamma} \right] \quad (3.4.8)$$

ii) The non-resonant term is dropped because its contribution becomes negligible as the resonant condition is approached.

$$\alpha_{\sigma\rho} = \sum_V \left[\frac{\langle F | \mu_p | V \rangle \langle V | \mu_\sigma | I \rangle}{E_V - E_I - E_L - i\Gamma} \right] \quad (3.4.9)$$

iii) The Born-Oppenheimer approximation is invoked to separate the electronic and vibrational component. The assumption is made that the electronic transition occurs well before any nuclear rearrangement can take place.

$$\langle F | \mu | V \rangle = \langle f | g \rangle \langle \mu | e \rangle \langle v \rangle = \langle f | M | v \rangle \quad (3.4.10)$$

$$\alpha = (g | \mu | e) (e | \mu | g) \sum_v \frac{\langle f | v \rangle \langle v | i \rangle}{E_v - E_i - E_L - i\Gamma} \quad (3.4.11)$$

iv) A Taylor series is used to expand the electronic part of the wavefunction in nuclear displacement. The expansion is truncated after the first term since,

in most cases, there are 3 orders of magnitude separating the first and the second term of the expansion series.

- v) It is assumed that a linear transition occurs between the potential surfaces of both the ground and the excited state (Franck-Condon approximation). In other words, there is no nuclear displacement before the end of the transition.

$$\alpha = M^2 \sum_v \frac{\langle f|v \rangle \langle v|i \rangle}{E_v - E_i - E_L - i\Gamma} \quad (3.4.12)$$

Finally,

$$\sigma_R = 5.87 \times 10^{-19} M^4 E_s^3 E_L \left| \sum_v \frac{\langle f|v \rangle \langle v|i \rangle}{E_v - E_i - E_L - i\Gamma} \right|^2 \quad (3.4.13)$$

$$\sigma_A = \frac{4\pi^2 e^2 M^2 E_L}{3 \hbar c n} \sum_v \frac{|\langle v|i \rangle|^2}{[E_v - E_i - E_L + E_0]^2 + \Gamma_e^2} \quad (3.4.14)$$

with all the energy terms and Γ_e in cm^{-1} , M in $\text{\AA}$ and σ in $\text{\AA}^2/\text{molecule}$.

Equation 3.3.14 shows the corresponding absorption cross-section with the same level of approximation. It is pointed out that Γ_e in an absorption spectrum will manifest itself by broadening the band without changing the integrated intensity. In Raman spectroscopy, the amplitude of Γ_e will take some of the intensity away from the real component into the imaginary part of the polarization. The resulting Raman cross-section is decreased.

In this simplified case, Equation 3.4.13 can be rearranged to

$$\sigma_R = \frac{R A}{(E_v - E_L)^2 + \Gamma_e^2} \quad (3.4.15)$$

where R is a constant and A represents the Franck-Condon overlap. The experimental excitation profile is a plot of Raman intensities as a function of the laser frequency ν_0 . Equation 3.4.15 can be fitted to the experimental intensities by adjusting the parameters ($R A$), E_v and Γ_e . To test the validity of the assumptions, these parameters should be introduced in Equation 3.4.14 to fit the measured absorption spectrum.

In cases where experimental data do not fit this basic sum-over-states model, it becomes appropriate to relax some of the approximations to identify the additional effects. The modifications most frequently considered are:

- i) contributions from other coupled modes (multidimensional Franck-Condon couplings);
- ii) mixing of electronic states (Herzberg-Teller intensity borrowing mechanisms, Albrecht's B term);
- iii) thermal effects, and
- iv) inhomogeneous broadening.^{28,80}

The expression to evaluate the Raman intensities of a large molecule becomes more and more elaborate as the number of normal modes of vibrations increases. In most cases, the sum-over-state development includes 2 or 3 modes to keep the basis

sets at a manageable size. Other theories have been developed to deal with the contribution of multiple modes.

B. Time-Dependent Theory: An alternative method is derived from the time-dependent perturbation theory, but an equivalent explanation is possible starting from the sum-over-states formulation and the latter is more intuitive.²⁸ The time-dependent method has been developed mainly to deal with multimode Raman calculations.²⁷ The main precept arises from the equivalence between the absorption and emission of a photon and the interaction of the wavefunction of an energy level with time-dependent potentials. It is stressed that the method is not a time-resolved description of the scattering event, but rather follows the evolution in time of a vibrational state i under the influence of a Hamiltonian operator which varies over time.

Using this time-frame viewpoint it becomes possible to calculate both the absorption and Raman spectra even with anharmonic excited state surfaces. The principal mathematical manipulation is to write the denominator of the Raman polarizability as a half-Fourier transform which represents the overlap between the propagating wavepacket $i(t)$ and the final state f in the Raman process.

$$\alpha_{i-f} = \frac{1}{\hbar} \int_0^\infty \sum_v \langle f|v \rangle \langle v|i \rangle \cdot \exp \left[\frac{-i(E_v - E_i + E_0 - E_L - i\Gamma)}{\hbar} \right] dt \quad (3.4.16)$$

The bandshape is determined by the dependence of $\langle f|i(t) \rangle$. The corresponding absorption cross-section is written as a full Fourier transform²⁸ of the

energy denominator.

$$\sigma = \frac{4 \pi e^2 M^2 E_L}{6 \hbar^2 c n} \int_{-\infty}^{\infty} \langle i | i(t) \rangle \exp \left[\frac{i (E_L + E_i) t}{\hbar} \right] e^{-\Gamma t / \hbar} dt \quad (3.4.17)$$

Both sum-over-states and time-dependent description give the same information. The nature of the problem studied (i.e. number of modes, accuracy of the function) determines which formalism is most appropriate to treat the problem. In the sum-over-states viewpoint, resonance Raman intensities depend on the excited-state potential surface. In the time-dependent framework, the excited state potential directly determines the evolution of the vibrational wavefunction during the interval between the disappearance of the incident photon and the creation of the scattered photons.²⁸

C. Transform Theory: Resonance Raman intensities have been calculated by transform methods in both the sum-over-states^{96a} description and the time-correlator formalisms⁹⁶. The basic premises of the transform theory relies on the Kramers-Kronig relationship between the optical absorption cross-section and the resonance Raman amplitude. As a result, theoretical resonance Raman excitation profiles may be calculated from the experimentally measured absorption spectrum.

The optical theorem provides a relation between the real and the imaginary part of the complex refractive index.

$$N(\nu) = n(\nu) - (i/4\pi \nu) A(\nu) \quad (3.4.18)$$

whose $n(\nu)$ is the real (or ordinary) index of refraction and A is the absorption

coefficient (the imaginary part). From the imaginary part of the complex index of refraction arises the absorption band while the real part is responsible for the dispersion spectrum. The parameters $n(\nu)$ and $A(\nu)$ are related by a Kramers-Kronig relations.

The deep theoretical relationship has been described by Hizhanyakov and Tehver²⁵ for Rayleigh and Raman scattering in the late 1960s but has been applied only recently to the understanding of the resonance Raman effect. The imaginary part of the amplitude for Rayleigh (or elastic scattering) is proportional to the absorption cross-section.⁶⁴ Furthermore, the real and imaginary parts of the scattering amplitude are Kramers-Kronig relatives of each other.¹⁰⁰ For Raman (or inelastic) scattering, this direct relationship does not hold, but a similar one has been derived.¹⁰¹

At the simplest level of approximation, the basic transform relation can be expressed as¹⁰²:

$$\alpha_{0-1}(E_L) \propto S_1^{1/2} [A_0(E_L) - A_0(E_L - E_{if})] \quad (3.4.19)$$

where $\alpha_{0-1}(E_L)$ is the resonance Raman amplitude (polarizability) for the 0-1 transition, E_L is the laser energy, E_{if} is the vibrational energy for the Raman mode, and $S_1 = \Delta^2/2$ for the Raman mode. The quantity, Δ , is a dimensionless quantity describing the shift from the origin or the ground and excited state harmonic surface of the given mode. The function $A_0(E_L)$ is defined by

$$A_0(E_L) = P \int_0^\infty \frac{\sigma(E)}{E(E-E_L)} dE + i\pi \frac{\sigma(E_L)}{E_L} \quad (3.4.20)$$

where $\sigma(E)$ is the optical absorption cross section at energy E , and P denotes the principal part of the integral, i is the imaginary unit. $A_0(E_L - E_{if})$ is the same function except that it is shifted by the equivalent of one vibrational quantum of the mode to higher energies.

The first term in Equation (3.4.20) represents one of the Kramers-Kronig relations between $A_0(E_L)$ and $\sigma(E)$, thus establishing their causal relationship. In other words, $A_0(E_L)$ is simply the Hilbert transformation of $\sigma(E)$. The complete expression for the resonance Raman cross-section for the fundamental of the mode can now be written as

$$\sigma_{0 \rightarrow 1} = \frac{E_L E_s^3 n^2}{2 \hbar^2 c^2 \pi^3} S_1 |A_0(E_L) - A_0(E_L - E_{if})|^2. \quad (3.4.21)$$

In the above expression, all energies are in cm^{-1} and the σ' is in $\text{\AA}^2/\text{molecule}$. From Equation 3.4.21, the resultant shape of the Raman excitation profile depends directly on the shape of the absorption band and the mode frequency. This is to be expected when the Kramers-Kronig relationship is invoked because the absorption function and the Raman profile functions are assumed to be causal functions. Equation 3.4.21 indicates that the absolute scaling of the Raman profile depends on S which explicitly depends on the excited state displacement Δ . The value of Δ can be obtained directly from the cross-section of absorption and Raman mode at a given excitation energy, E_L .

The transform method provides a basic means for calculating "first-order" resonance Raman profiles. The restrictions mentioned below may be relaxed and

Equation 3.4.21 may be modified to include them. In the simplest case, however, the following assumptions are made:

- i) no inhomogeneous broadening;
- ii) a single electronic state;
- iii) constant transition length (Franck-Condon approximation);
- iv) no frequency changes or Duschinsky rotation involving the mode(s) that are undergoing a vibrational transition in the Raman process (*i.e.* linear electron-phonon coupling), and
- v) no Herzberg-Teller mechanism.

The principal-value integral in Equation 3.4.20 may be evaluated analytically from a digitized absorption spectrum as described by Chan and Page⁹⁶. Although it is possible to calculate a Hilbert of a digitized function, the computer time is greatly reduced by conducting the Hilbert transform indirectly using fast Fourier transform, where the symbol $*$ represents a convolution.

$$F_{Hi} = \frac{-1}{\pi x} * f(x) \quad (3.4.22)$$

The Hilbert "filter" does not affect the amplitude of the spectral components but their phases are altered by $\pi/2$ positively or negatively according to the sign of x .¹⁰² Figure 3.3 present the intermediate steps to generate a Raman excitation profile from the digitized absorption spectrum, using a transform method.

Figure 3.3

Intermediate steps of the Transform method: a) the imaginary part of the equation is obtained by the square of the difference between the digitized absorption curve and the same curve displaced by the mode of interest, in this case 1189 cm^{-1} ; b) the real part is obtained by the same operation done on the Hilbert transform of the absorption spectrum; c) the Raman excitation profile is obtained by the sum of the real and the imaginary part; d) the REP is shown in comparison with the original absorption spectrum.

3.5 DERIVATIVE SPECTROSCOPY

Absorption bands are a physical representation of electronic energy levels. Vibrational and rotational energy levels make absorption bands broader than would be expected from the quantum changes of electronic states alone. In mixtures and/or for complex molecules, the absorption spectrum might be further complicated by the close proximity of several peaks with different bandwidths. Small features of the spectrum might easily disappear under the background and/or the noise.¹⁰³

Derivative spectroscopy was first introduced in 1955 as a means to resolve close spectral lines.¹⁰⁴ Derivative spectroscopy eventually evolved into an elegant analytical tool to be used to characterize qualitatively or quantitatively^{103,105,106} the different bands in a spectrum. Differentiation of a spectrum results in better resolution by sharpening broad bands and an amplification of smaller features. Consequently, it becomes easier:

- i) to determine the maximum (or maxima of close peaks) of a broad band;
- ii) to isolate shoulders, and
- iii) to resolve and to enhance weak signals.^{103,107,108}

For a Gaussian lineshape, the improvement in resolution for a second-order derivative has been estimated at 1.8 times and at better than 3.0 times for a Lorentzian lineshape.^{103,107} The price to pay for this increase in resolution, unfortunately, is a loss of information. The signal-to-noise ratio of derivative spectra deteriorates quickly

with the order of the derivative (4-10 times for the second order derivative).^{107,108} Moreover, the derivative spectra become increasingly complex as the number of extrema increase with the order of the derivative. One maximum of the initial curve produces $n+1$ extrema in the n^{th} order.^{107,108}

Differentiation of the analytical bands can be done either by digital calculation (after data collection) or directly by electronic circuitry (data acquisition of derivatives).^{103,105,107,108} The derivation gives the slope or the curvature of the intensity distribution of a spectrum. The transformation makes the curve sensitive to changes in intensity with respect to wavelength.^{105,107,109} According to the familiar law of Beer-Lambert, the change in intensity, I/I_0 , is described by:

$$\frac{I}{I_0} = e^{-C l \epsilon} \quad (3.5.1)$$

where C is the concentration of the sample (mol/L), l is the sample thickness (cm) and ϵ is the molar extinction coefficient ($\text{L-mol}^{-1}\text{-cm}^{-1}$). Assuming a constant I_0 , the first and second derivative of the intensity with respect to the wavelength are:

$$\frac{\frac{d I}{d \lambda}}{I_0} = -C l \frac{d \epsilon}{d \lambda} \quad (3.5.2)$$

and

$$\frac{\frac{d^2 I}{d \lambda^2}}{I_0} = C^2 l^2 \left[\frac{d \epsilon}{d \lambda} \right]^2 - C l \left[\frac{d^2 \epsilon}{d \lambda^2} \right] \quad (3.5.3)$$

When ϵ is linearly proportional to the concentration, its derivative, $d\epsilon/d\lambda$, is equal to zero but extremum values are formed in the neighbourhood of inflection

points. Inflection points, therefore, are more readily identified in derivative spectra.^{103,104,105}

The advantage of the 2nd derivation method becomes particularly obvious on the analysis of the position of overlapping bands. The composite absorption band, I , can be expressed as a sum of Gaussian curves where, I_1 and I_2 vary as a function of wavelength and represent absorption bands with maximum at λ_0 and λ_0 shifted by $\Delta\lambda$ respectively. The profile of the band is described as:

$$I = I_1(\lambda_0) + I_2(\lambda_0 - \Delta\lambda) \quad (3.5.4)$$

The individual bands are defined as:

$$I_1(\lambda) = I_{1\max} \cdot \exp(-(\lambda_0 - \Delta\lambda)/2B^2) \quad (3.5.5)$$

where $I_{1\max}$ is the ordinate maximum of the peak and B is the band half-width. The curve for I_2 is described in a similar manner. The derivative of the composite band can be taken simply as a sum of the individual derivatives. Characteristic profiles of individual and composite Gaussian curves are shown in Figure 3.4 along with their first and second derivatives.

Good separation of near-by bands is expected when the difference between the maximum abscissa is greater than the sum of the half-bandwidth divided by a factor

$$\Delta\lambda > (B_1 + B_2)/\tau \quad (3.5.6)$$

where r equals 1.59 for a Gaussian curve and 3.09 for a Lorentzian.¹⁰⁵ O'Haver *et al.*¹⁰⁶ provided a detailed analysis of quantitative measurements of overlapping bands. They compared the results of using 2nd derivative spectroscopy to estimate the parameters of two overlapping spectral bands for a various range of band heights, relative maxima positions and band widths.

Because the 2nd derivative method is useful to determine the peak position and give its approximate bandwidth, it could be of assistance prior to deconvolution. Derivative spectroscopy is not yet developed to the point that it will displace powerful and efficient methods such as deconvolution, least-square, or resolution enhancement,¹⁰⁶ but its simplicity and its speed still remain attractive for a first analysis of a spectrum.

Figure 3.4

Characteristic Profiles of Derivation of a) a Gaussian curve, 1st and 2nd derivative b) a mixture of two Gaussians of equal intensity c) a mixture of two Gaussians of different intensity The number 0, 1, 2 represents the order of the derivation.

4. EXPERIMENTAL SECTION

4.1 INTRODUCTION

This chapter is divided into several sections outlining the steps executed in performing Raman spectroscopy of Chl *a* in solution. The chapter starts with the preparation of the Chl *a* samples (Section 4.2 - 4.4), followed by the description of the sample cells and the flowing cell system in Section 4.5. Section 4.6 describes the instrumentation used to record the Raman spectra executed by a nanosecond pulsed laser. Section 4.7 is devoted to the description of both the hardware and software used in automated data acquisition. Other relevant experimental conditions are mentioned in Section 4.8.

4.2 PIGMENT EXTRACTION

All procedures were carried out in the dark or in dim light to prevent photo-degradation.^{109,112,113} Chl *a* was extracted and isolated from spinach leaves using the method outlined by Omata and Murata.¹¹⁰ The procedures are briefly described below. The extraction of about 1 kg of spinach leaves was carried out each time.

Fresh spinach leaves removed from their stems and midribs were first shredded into small pieces ($\approx 1 \text{ mm}^2$). Approximately 600 ml of shredded spinach pieces was put into a 1 L beaker and scalding distilled water was poured onto the leaves until they

softened (1 - 2 minutes). The excess water was drained and squeezed out with paper towels. The spinach pieces were then immersed in a minimum amount of cold acetone and placed in an ice bath in the dark until most of the pigments went into solution (2 - 4 hours). The leaf material was then removed after a qualitative analysis of the solution colour,* to evaluate the chlorophyll content of the extract. The pigments were precipitated by adding 1-4 dioxane (60 ml/500 ml acetone), followed by distilled water (100 ml/500 ml acetone). The solution was left undisturbed to allow a green precipitate to form (4 - 8 hours). The colloidal precipitate was separated by vacuum filtration on a coarse sintered glass filter. The solid was then recovered by washing the glass filter with ethanol and the solvent was immediately evaporated under vacuum. The resulting residue was washed with small aliquots of hexane to separate soluble chlorophylls from less soluble pigments (e.g. water-soluble carotenoids). The hexane solution was then dried under vacuum until a black amorphous solid was obtained.

4.3 CHLOROPHYLL *a* PURIFICATION

At this point, the extract contained a wide variety of compounds apart from the required Chl *a*.¹⁰⁹ Chl *a* was purified by column chromatography in two separate steps:

- i) removal of carotenoids from the crude pigment extract; followed by
- ii) isolation of Chl *a* from other chlorophyll pigments.

The chromatography columns (2 cm diameter) packed to a height of 3 - 4 cm

were water-cooled to keep the column material at about 10 °C. This helped to prevent bubbling owing to the gel nature of the column material and to preserve the pigments. The column materials¹¹¹, Sepharose-DEAE and Sepharose CL-6B, were purchased from Pharmacia Fine Chemicals. They were used after successive filtration and re-suspension in their respective series of solvents to prepare for gel filtration. The solvents used for Sepharose-DEAE were distilled water, 1 M sodium acetate buffer (pH 7), distilled water, and acetone. For the Sepharose CL-6B, distilled water, acetone, acetone/hexane (2:1 v/v), acetone/hexane (1:2), hexane/2-propanol (10:1), hexane/2-propanol (20:1) were used successively.

Small amounts of solid crude pigment extract were dissolved in 1 ml of acetone and applied to the DEAE Sepharose column. Pheophytins (grey band), and carotenoids (yellow-orange band) moved quickly with acetone at a flow rate of 2 ml/min. The adsorbed chlorophylls were then recovered with an acetone/methanol (10:3) mixture. The solvent was immediately evaporated under vacuum. This purification step was repeated as often as necessary (up to 3 times) until carotenoids could not be detected by thin layer chromatography (TLC) on Silicagel G (Merck). The TLC plates were developed in chambers that were pre-equilibrated for approximately 10 minutes with the developing solvent which was made of a mixture of hexane/acetone/*i*-butanol (90:5:5).

After this pre-purification step, the next task was to isolate Chl *a* from other chlorophylls or by-products. The pigment material (free of carotenoids) was dissolved in a small aliquot of hexane/2-propanol (20:1) and applied to a Sepharose CL-6B column. Hexane/2-propanol (20:1) was the eluent, at a flow rate of 1 ml/min. The first grey band to be eluted revealed the presence of pheophytin *a*. A small green band,

Chl *a'*, ran slightly ahead of Chl *a*¹⁰⁹; as much as possible, Chl *a'* was not collected. The same gel filtration was repeated up to 4 times and the results were checked by TLC and HPLC. The developing solvent for TLC was hexane/acetone/*i*-butanol (15:2:2). The HPLC chromatograms were obtained with a Waters Associates M-6000 Series using a silica column (46x220 mm, Brownlee). A one-step solvent program was used to monitor the separation of the chlorophylls (more particularly, Chl *a'*, Chl *a*, and Chl *b*). The program was run for 20 minutes and the absorbance was measured at 430 nm with a SF-770 Spectroflow U.V. detector (Schoeffel Instrument). Chromatograms obtained by HPLC enabled the measurement of the ratio of Chl *a'* and Chl *a*. As an observation, even when the measurements were taken immediately after the isolation of Chl *a*, the proportion of Chl *a'* in the final samples was higher (30%) than the value reported in literature (15%) for isomerisation over time.¹¹⁴ Samples isolated up to 12 months earlier were also analyzed by HPLC; 45% of the content was made of Chl *a'* but the samples seemed to have maintained their integrity otherwise. It was thought that the column material itself might cause epimerization.¹¹⁶ For that reason, it was difficult to estimate the actual proportion of Chl *a'* left in the purified samples. It must be kept in mind however, that the visible absorption spectra of Chl *a* and Chl *a'* are nearly identical.^{46,116} It was assumed therefore, that the spectral information obtained for this work was not significantly affected by the presence of the equilibrium between the epimers.

The purified Chl *a* was dried by co-distillation with 3 successive fractions of dry carbon tetrachloride and 3 fractions of dry hexane.⁴² The drying solvents were of spectral grade quality and kept over molecular sieves of 3 Å, 8 - 12 mesh (Aldrich) to remove traces of water. Deoxygenation of the solvents was accomplished by passing nitrogen (N₂) through a desiccant column (25 cm of Silicagel) and bubbling it

through the solvent for 15 - 20 minutes. The N_2 delivery tube was then raised above the solution, where the flow was continued until the cell was closed with a Teflon stopcock. The dried Chl *a* was kept in a sealed flask in a glove box under a constant overpressure of N_2 . The glove box was covered with a heavy thick cloth to minimize photodecomposition. Figure 4.1 provides a flow chart showing the major steps of the extraction and the purification of Chl *a*.

The purity of Chl *a* was also verified by absorption spectroscopy (Perkin-Elmer Lambda 4B UV/Vis spectrophotometer), as well as the chromatographic methods. The purity was established as described by Svec et al. by measuring the intensity ratio of the red and blue absorption bands (blue/red: $1.30 \pm .05$).⁴⁰

4.4 CHLOROPHYLL *a* SAMPLE PREPARATION

4.4.1 Chl *a* in Hexane

Chl *a* was dissolved in the appropriate amount of hexane to yield the desired concentration, which was estimated by absorption spectroscopy. Approximately 50 ml of solution was prepared to be used in the flowing cell. A small aliquot of the solution was kept in the dark glove box as a control to compare periodically with the irradiated solution. The points of comparison were the peak position and intensity of their absorption and fluorescence spectra. The extent of dimerisation was qualitatively evaluated from the 2nd derivative of the absorption spectra. This technique is described in further detail in the discussion of results.

4.4.2 Chl *a* in Collidine

Collidine (2,4,6,-trimethylpyridine) was purchased from Caledon (97% pure). The compound was dried over potassium hydroxide, then fractionated with barium oxide. These oxide and hydroxide react with impurities present in collidine to form salts that will be left behind after distillation. The desired fractions were collected and distilled again under reduced pressure.

To facilitate the transfer of Chl *a*, a stock solution of Chl *a* in hexane was prepared in the glove box. A 1 mm cell was filled up to a mark with an aliquot of the solution and the solvent was evaporated under a gentle flow of N₂. The cell was closed with a Teflon stopcock and transferred into the glove bag. To avoid contamination of the glove box with collidine, the last part of the sample preparation was done in a separate glove bag (AtmosBagTM, Aldrich). Collidine was added to the cell containing the dry Chl *a* up to the same mark. This also allowed the approximate concentration of chlorophyll to be determined in advance before final dissolution in collidine. After a suitable period of time (5 - 10 minutes), the absorption spectrum of the collidine solution was taken. The molar absorption coefficient was taken as that of pyridine ($\epsilon_{670} = 7.69 \times 10^4 \text{ L-mol}^{-1}\text{-cm}^{-1}$).⁴⁴

4.4.3 Method to Evaluate Chl *a* Content

Because of the small amount of compound used in preparing the chlorophyll solutions, it was not always convenient to use a micro-balance, nor possible to avoid error from the weight which results from the moisture on glassware. The following method was used to facilitate the estimation of the dry Chl *a* content in a flask. To the flask containing the dry Chl *a*, 25.00 ml of dried hexane was added. With a syringe (Hamilton), 0.050 ml of the hexane solution was drawn and transferred to a 10.0 ml

Figure 4.1

Steps for the extraction and the purification of Chl *a*.

The answers yes and no in between steps of this flow chart diagram indicate the presence or absence of contaminants.

volumetric flask. The syringe was rinsed 3 - 4 times with hexane to ensure a complete transfer of the solution. The solvent was evaporated under a flow of N_2 . The 10.0 ml volumetric flask was filled to the mark with diethyl ether (for which the absorption coefficient of Chl *a* as a monomer is known). The absorbance was measured and the amount of Chl *a* was obtained from the calculation listed in Appendix A.

4.5 CELL DESIGN AND ASSEMBLY

The sample cells were designed to minimize decomposition caused by oxygen and long laser light exposure. A flowing cell system was used for samples that required long laser exposure when frequent change of solution was not practical

4.5.1 Sample Cells

All the square cells used for the experiments were made of quartz polished on at least 2 sides and fitted with Teflon stopcocks. A flowing cell was custom-made by Hellma. The outside dimensions were of a square 1 cm cell but the inside dimensions were 2x10x50 mm (see Figure 4.2). The cell was made of quartz polished on 4 sides and opened at both ends with 2 quartz tubes.

4.5.2 Flowing system

A low-flow diaphragm pump (Cole-Parmer 7088-48) was used to pump the chlorophyll solution in a closed-loop system. All wetted parts of the pump were covered with Teflon fluorocarbon resin to avoid contamination of the solution. The solution flowed through Teflon tubing and fittings that were hermetically sealed. A voltage controller (Cole-Parmer 2530-25) was used to adjust the flow rate to

Figure 4.2

Schematic diagram and dimensions of flowing cell.

2 ml/min. The reservoir was made from a 50 ml pear-shaped flask fitted with a screw cap lined with Teflon. A gas-bubbler was modified to allow solution intake and return to the reservoir. The glass tubing was designed so that gas bubbles were not being visibly trapped in solution and carried through the system (where they could have caused some undesirable scattering of the laser beam).

A polyethylene glove bag (Aldrich), with a constant overpressure of N_2 , enclosed the pump, the reservoir and all Teflon tubing and fittings, to prevent the slow diffusion of oxygen in solution. An inlet to the glove bag was cut to accommodate an electrical cable from the power supply/speed controller to the pump. The flowing cell was put in the optical arrangement and was connected to the pump by Teflon tubing and fittings. Two other ports of the glove bag were cut and sealed tightly around both ends of the flowing cell. The efficiency of the flowing cell system was tested by monitoring a Raman signal for a period of time equal to a complete scan. No change in the intensity of the Raman signal was observed.

4.6 INSTRUMENTATION

A schematic diagram representing the nanosecond Raman system is illustrated in Figure 4.3. The various components used to observe and record Raman spectra consisted of lasers, a sample device, a monochromator and a detection device. The equipment is described in this section.

4.6.1 Lasers

The properties of lasers have been found invaluable in the development of Raman spectroscopy. They provide a high flux of collimated photons of a given wavelength range. As a consequence of stimulated emission, phase coherence is an important characteristic of lasers.¹¹⁷ It means that all photons within the beam have the same direction and phase relationship. The monochromaticity is limited to the excited state transition induced in the lasing material. The wavelength range is often narrow enough to excite molecules selectively in a mixture of molecules having slightly different absorption spectra.

A nitrogen laser (Lambda Physik M2000) (337 nm) was used to pump a dye laser (Lambda Physik F12000) at a repetition rate of 30 Hz. The laser pulse width was 4 ns. The two-stage tunable dye laser is capable of providing wavelengths over the visible range from 380 to 700 nm using a range of laser dyes. The dyes that were used are listed in Table 4.1. The maximum average power obtainable from the dye laser is 5 mW corresponding to a peak power of 2×10^4 W or 10^{13} photons per pulse. However, the conversion efficiency of the dye in the blue region varied widely with the dye and the wavelength of interest.

Performing Raman spectroscopy with pulsed lasers has advantages and disadvantages. Although pulsed lasers are a useful source of intense monochromatic light, the high flux of photons increases the probability of generating excited states and/or by-products which could result in unwanted contributions to the observed Raman spectrum. In order to minimize these two disadvantages, the following precautions were taken. First, saturation of the S_1 level of chlorophyll for all Raman measurements was made the same by using laser light with at least 0.8 mW of power.

Figure 4.3

Schematic diagram of the nanosecond laser Raman system.

M1 to M6: mirrors

CL: cylindrical lens

COLL: collecting lens

FP: fast photodiode

MD: monochromator drive mechanism.

Second, a flowing cell was used which contained a large volume of sample (50 ml), thus minimizing photodecomposition. These effects were checked by ensuring that the Raman intensity varied linearly with the photon flux from the laser.

Table 4.1. Laser Dyes Pumped with N₂ Laser and Their Experimental Characteristics.

Wavelength range (nm)	Dye ^a	Concentration (mM)	Solvent	Power ^b (mW)
404-410	DPS	1.0	Dioxane (5% Methanol)	2.0-4.1
414-425	Popop	1.0	Dioxane	2.6-3.0
416-435	Stilbene 3	0.5	Ethanol	1.0-4.0
418-425	LD 423	3.0	Ethanol	1.1-2.5
428-450	Coumarin 120	2.0	Ethanol	2.5-3.0

^a These laser dyes were all purchased from Lambdachrome except for LD 423 which was obtained from Exciton.

^b Power values corrected for wavelength dependency and superradiance.

4.6.2 Sample Device

Optical alignment consists of the placement of collecting lenses, sample, and other optical components used to direct the laser beam onto the sample. The position of all optical components with respect to the detection system is especially critical to compensate for the low cross-section of Raman signals (and with chlorophyll samples more particularly). First, the optical components were raised to the proper height of the monochromator and secured on optical bars. The main characteristics of the

alignment were:

- i) the distance for the coupling of the two collecting lenses at the entrance of the monochromator;
- ii) the distance of the front optics from the sample;
- iii) the distance of the cylindrical lens from the sample, and
- iv) the position of the mirrors used to direct the laser beam.

The two mirrors and the cylindrical lens were mounted on their respective gimbal mounts for fine adjustments. The light beam provided by the dye laser was dispersed with a Pellin-Brocca prism (to disperse the superradiance) onto a small mirror (1.5 cm diameter), through an iris, and onto two other mirrors closer to the sample cell (see Figure 4.3). In order to illuminate a larger volume of sample, a cylindrical lens was used to transform a light beam point into a stripe of light. A small mirror (1x3 mm) — glued onto a wire which was connected to an aluminum disk mounted in a gimbal mount — was placed between the cylindrical lens and the sample to direct light onto the sample. The scattered photons coming from this 180° geometry were collected and focused by using two collecting (spherical) lenses placed in front of the monochromator.

Raman intensities obtained in the resonance region are often subject to distortion as a result of the sample's re-absorption of the scattered light. This happens, for example, when a sample of chlorophyll is excited with laser light at 408 nm ($24\,510\text{ cm}^{-1}$) and the scattered light at 429 nm ($23\,311\text{ cm}^{-1}$) — the 1189 cm^{-1}

Figure 4.4

Position of the optical components used to generate and collect scattered photons from Chl *a* solution in a 180° geometry.

scattering shift — falls in the absorption region of the sample. The 180° geometry was chosen because this configuration minimized the re-absorption effect and maximized the collection efficiency for the scattered photons.¹¹⁸ In this arrangement, most of the laser light that was reflected by the cell wall was shadowed by the small mirror and a large portion was collected and focused into the monochromator. Consequently, it precluded the collection of Raman light below 650 cm^{-1} . Fortunately, the region of interest for this study was outside this limit.

In addition to the use of a 180° geometry, the two following precautions were also taken to minimize the re-absorption effect. First, the concentration of the solution was adjusted so that for a given path length (typically 1 mm), the illuminated volume of the sample was nearly the same for all wavelengths. The cross-section of the molecule (concentration) was adjusted to let a sufficient number of photons illuminate the whole sample volume even near the Soret absorption conditions. Second, the Raman intensity of a solvent band in the vicinity of a Raman band of the solute was taken as an intensity monitor for the possible wavelength-dependent change. The ratio of the two Raman intensities was used as the corrected value for resonance enhancement. This procedure has the added advantage of correcting not only for re-absorption effects but also for other wavelength-dependent effects (e.g. detector efficiency, grating efficiency).^{28,78}

4.6.3 Monochromator Spectrometer

The function of a monochromator is to segregate wavelengths so that a single wavelength can be passed for analysis. The Jobin Yvon Ramanor HG2 is a double

monochromator with concave holographic gratings ($(110)^2 \text{ mm}^2$), 2000 grooves/mm, a 1 m focal length, and F/8 aperture. The monochromator has a Littrow configuration which means that the entrance and exit slits are horizontal and mounted in a plane parallel to the rotation axis. There are four (4) sets of slits which are independently controlled by a stepper motor and by re-transmitting potentiometers. The slits can have a width of up to 2 mm with 1 μm definition. The mechanical range of the monochromator is 25 000 to 12 800 cm^{-1} , but the optimum visible range is 22 720 to 13 500 cm^{-1} . The stray light rejection is estimated at 10^{-14} at 20 cm^{-1} from the Rayleigh line. The resolution can be better than 0.5 cm^{-1} at 514.5 nm depending on experimental conditions (e.g. small slitwidths, scan speed). The wavenumber scale is read from a cosecant bar which translates linearly the grating orientation into wavenumbers.

4.6.4 Detection System

The detection of the Raman signal is achieved by the transformation of photons into electronic current by a photomultiplier tube. The signal is amplified and filtered with a boxcar gated averager. These two pieces of equipment are described below.

A. Photomultiplier Tube: The photomultiplier tube (PMT) is a detecting device that is very sensitive to low intensity light signals.¹¹⁹ The detection and amplification of the photons achieved with a PMT can be sub-divided into three elements: a photocathode, a system of dynode multipliers, and a collector.¹²⁰ The photons incident on the coating of a photocathode cause the emission of photoelectrons. The coatings (usually containing elements from group I and/or V of the periodic table) are selected in relation to their spectral sensitivity and photoelectric efficiency. The photoelectrons impinging on the first dynode produce a number of secondary

Figure 4.5

Plot of intensity of the Raman signal of the 1460 cm^{-1} band of hexane as a function of high voltage for two PMTs. The readings from the RCA 8850 and the RCA 3134A are represented by squares and crosses respectively.

Figure 4.6.

Plot of the signal-to-noise ratio as a function of high voltage for two PMTs. The readings from the RCA 8850 and the RCA 3134A are represented by squares and crosses respectively.

Figure 4.7

Plot of the Raman signal intensities as a function of laser power obtained for two high voltages applied on the RCA 8850 photomultiplier tube. The standard deviation is represented by the error bars on the data points.

electrons. This step corresponds to an amplification of the signal. The process is repeated through a series of dynodes and the resulting photocurrent is collected at an anode. The output signal is a measure of the radiation intensity. PMTs offer very high gain, high sensitivity and very low background noise. Theoretically, PMTs' performance are near ideal if optimized by using the range of accessories to improve the tube performance. The accessories are: voltage divides, power supplies, cooled housing and amplifier discriminator. PMTs are very sensitive to change in high voltage and are prone to saturation.¹²¹

In order to determine the characteristics of the PMT used for the collection of Raman data, the following experiments were carried out. Two PMTs were considered, a RCA 31034A and a RCA 8850, since their respective response curves were in the region of interest (350 - 500 nm). First, an experiment was conducted to establish the relationship between the high voltage applied on the tube and the increase in signal intensity of a Raman signal (1460 cm^{-1} line of pure hexane). Then, the relationship between the light intensity and the response was investigated. The signal-to-noise ratio (S/N) was taken as a measure of the quality of the amplification since it is only relevant if the noise is not also amplified. A light signal was measured as the difference between the top of a Raman band and the baseline of the spectrum. These data were an average of 260 data points collected at a rate of 1 point/s for each position of the monochromator. Thirty (30) points were already averaged by the boxcar for each point collected.

In Figure 4.6, the intensity of the Raman signal is shown as a function of high voltages on the PMT. As the voltage increases, so does the signal and the S/N also improves before it reaches a plateau at 1600 V (Figure 4.7). However, the

RCA 8850 tube provided a larger amplification of the signal. The RCA 8850 was ultimately chosen since, in spite of the more limited range of wavelength detection, it provided the best sensitivity. This PMT also demonstrated linear amplification at two high voltages as a function of laser flux (photons/s), as illustrated in Figure 4.8. The error bars in this last figure represent the standard deviation of the measurements. They indicate the deterioration of the S/N as the voltage is increased. To reach a compromise between gain and uncertainty of the measurements, the high voltage applied on the RCA 8850 was set at 2000 V.

B. Gated Integrator: Experiments based on pulsed lasers as the excitation source require signal averaging of fast signals. A boxcar averager offers two major advantages: it improves S/N and is compatible with fast signals. The Stanford Research Systems SR250 Gated Integrator and boxcar averager consists of a gate generator, a fast gated integrator, and exponential averaging circuitry.

Instead of the output pulse provided by the N₂ laser's thyatron, a fraction of the dye laser beam was converted into an electrical signal by a fast photodiode to trigger the time base of the gate generator. The intrinsic trigger-to-sample residual delay of the boxcar was typically 40 - 70 ns. Since sampling is needed nanoseconds after the trigger, the photomultiplier tube signal output was sent through a delay box (EG&G DB263) before being fed to the boxcar signal input. A moving exponential average is the traditional averaging technique. The average converges to the mean value of the signal while the noise averages to zero. Recording of each fluorescence and Raman data point was done after averaging 30 and 100 samples respectively.

4.7 DATA ACQUISITION

There are several advantages associated with computer-assisted control of experiments:

- i) a limited and consistent interaction with the instrumentation makes it less tedious for the operator;
- ii) a systematic acquisition of data in digital form simplifies the subsequent data manipulations;
- iii) an external monitoring of experiments allows, for example, the detection system to be kept in a dark environment or limits laser light exposure.

Coupling of a computer to a Raman spectrometer for data acquisition requires both hardware and software. Both are described below.

4.7.1 Hardware

There were few modifications made to adapt the spectrometer for digital data acquisition. The conversion of the analog signal to a digital record was achieved by using an interface module (Stanford SR245, hereafter called SR245). The output of the boxcar averager was integrated for 400 μ s. The laboratory computer was connected to the SR245 with a RS232 cable configured as illustrated in Figure 4.9. On the front panel of the SR245, there are 2 digital and 8 analog ports that can be programmed as input or output. The digital ports have TTL logic levels of 8-bit resolution. The analog ports have a range of ± 10.24 V with 13-bit resolution (2.5 mV). The maximum A/D conversion rate is 2 KHz. The SR245 is remotely programmable (with

most common computer languages) via either the RS232 or GPIB rear panel. The computer ASYNC port COM1 was set at the same baud rate, parity, and number of stop bits as those selected by the dip switches of the SR245 (see Table 4.2). The commands most frequently used with the SR245 are listed in Table 4.3.

The Ramanor HG2 consists of a monochromator and a control console for wavenumbers and slits. The electronic components are assembled in a cabinet. The monochromator can be controlled, either manually by depressing keys located on the control console or by remote control by voltages sent to the control console. For the automated data acquisition, the commands from the computer were sent to the computer of the Ramanor HG2 via the SR245 interface module. Pin configuration of the 37-pin connector of the Ramanor HG2 and the output ports of the SR245 are listed in Table 4.4.

Table 4.2. Configuration switch settings of the interface module SR245 for an RS232 and a ASYNC port configured as COM1:19200,n,8,2.

Bit	Setting	Explanation	COM1 Setting
8	OFF	RS232 operation	
7	OFF	disables Echo	
6	ON	2 Stop Bits	2
5	OFF	disables Parity	8 data bits no parity
4	OFF	Parity's Parity	
3	ON	19.2 Kbaud	19200 baud
2	ON		
1	ON

Figure 4.8

Configuration of the RS232 cable to link the serial port of the computer to the RS232 port of the interface module.

Table 4.3. The most commonly used SR245 commands.

Command	Description
I_n $n=0-8$	—Designates the first n analog ports as inputs, the remainder become outputs. Default is I8.
T_n $n=1$ to 32767	—Designates every n^{th} pulse at sync 2 as a trigger.
W_n $n=0-8$	—Introduces a delay of approximately $n \times 400 \mu\text{sec}$ before sending each character over the RS232, allowing slow peripherals to keep up.
$S_n=x$ $n=1-8$ $x = \pm 10.24 \text{ V}$	—Sets the analog port n (which must be designated as an output) to value x .
$SB_{n=m}$ $n=1,2$ $m=0,1$	—Designates digital bit n as output and sets its value to TTL low if $m=0$, high if $m=1$.
;	—Separates commands.
\$	—End of command string.

The number of steps to be taken by the stepper motor was set by successive TTL pulses (1 pulse = 0.1 cm^{-1}) coming from the digital port (sync 2). Voltages sent from SR245 analog port 8 set the direction of the scan (+5 V to increase, -5 V to decrease in wavenumbers). The digital port sync 1 was configured as an input to synchronize data acquisition with a trigger from the busy output of the boxcar during an experiment.

Table 4.4. Pin configuration for the connection between the SR245 ports and the Ramanor HG2 computer^a

Pin #	Code	SR245 ports	Function
2	clock	sync 2	—stepping motor control 1 pulse = 0.1 cm^{-1}
3	direction	port 8	—scanning direction 1 TTL = increasing ν 0 TTL = decreasing ν
4	auto	output	—remote control
5	stop +	output	—upper mechanical boundary 0 TTL = stop
28	ground		—ground

^a The other pins from the 37-pin connector were shorted out.

4.7.2 Software

During an experiment, a laboratory computer was responsible for:

- i) managing the acquisition, averaging and storing of data in memory, and
- ii) controlling peripheral devices.

Afterwards, the computer was used to perform data manipulation including some means of display and making permanent records.

The role of the computer (see specifications in Table 4.5)-was primarily to control the stepper motor of the monochromator. A stepper motor consists of a gear-like inner rotor surrounded by three or more gear-like stators. The rotor tends to align itself with the stator which is actuated with an electro-magnet as a result of magnetic induction. By switching current sequentially between the stator magnets, the rotor can be stepped in a rotary or back and forth motion. Controlling the stepper motor was simply a matter of sending voltages to the appropriate stator magnets at the proper times.¹²¹ The Ramanor HG2 is equipped with a 4 - phase stepping motor drive.

Table 4.5.

Raven Computer Features (IBM PC clone manufactured by the Science and Technology Workshop of Carleton University).

	640 Kbytes of RAM
	8087 math co-processor
	20 Mbytes hard disk
2	Mbytes J-RAM disk (volatile)
1	5 1/4" floppy drive
2	ASYNC I/O ports configured as COM1 and COM2
1	parallel port configured as LPT1
	Hercules Monochrome Graphics Card
	Monochrome monitor

4.7.3 Software Programs

All the following programs were part of a DOS batch file and were executed in

sequence at the touch of one key or could be called individually. Table 4.6 lists all the programs used and their functions. The programs are described in more detail below. The source files for the programs written by the author appear in Appendix C.

A. **SID.EXE:** A scan identification program, **SID.EXE**, was a FORTRAN program that prompted questions to be answered in order to:

- i) record the manual readings;
- ii) calculate parameters necessary to the execution of JY265;
- iii) provide a hard copy of the scan parameters;
- iv) serve as a checklist before a series of scans.

B. **RESET.EXE:** The computer program **RESET.EXE**, written in FORTRAN, was used to rewind the monochromator after removing the backlash by stepping an extra 100 cm^{-1} past the desired position and scanning down slowly to the starting position.

C. **JYUSER.FOR and JY265.EXE:** A software package (**SR265.EXE**) supplied by Stanford Research Systems was used for data acquisition and peripherals control. This FORTRAN program calls three subroutines (provided in source code **USER.FOR**) which may be modified by the user to provide a variety of functions during scans. The subroutines interact with the peripherals by sending SR245 commands (see Table 4.3). Commands are sent to the SR245 via a subroutine (**MAC12**) which sends them over the computer's COM1 port to the SR245. The subroutine **JYUSER.FOR** was written to customize data acquisition by modifying two

subroutines of USER.FOR. For this application, JYUSER.FOR controls the number of steps, the speed, and the direction of a stepper motor during and after a scan.

The subroutine EACHPT, called at each point in scan, sends commands to increment the stepper motor. Data points are collected at 0.1 cm^{-1} intervals for Raman and 3 cm^{-1} for fluorescence. The computer keeps track of the position of the monochromator so that it is possible for the subroutine EOSCN, called after each scan, to send commands to rewind the monochromator to the initial position after removing the backlash.

With these subroutines, it is possible to run and to average up to 5 scans without resetting the spectrometer. The modified subroutine JYUSER.FOR, once compiled and linked with the other source codes of the SR265 software package, was named JY265.EXE. One of the SR245 commands was accessed directly during the initialization of the parameters and was not included in the modified subroutine. At the prompt, (" Other SR245 commands?, At Each Point: ") T30 was entered to instruct the computer to accept a voltage reading every 30^{th} pulse at sync input in order to take advantage of the averaging capability of the boxcar. In this way, data acquisition was carried out at a rate of 1 s/point for a 30 Hz trigger. After the completion of the scan, the display of the spectra could be enlarged to fill the monitor screen and could be printed for a hard copy. A recorder was also used simultaneously with the data acquisition to monitor the scan and as a back-up.

The data files, which have the filename extension, ".DAT", were unformatted, binary, sequential files. They were not ASCII files. This was done to minimize the amount of space taken by the data files and to minimize the time to

access the files. The structure of the data files is described in the listing of program MUNCHBIN in Appendix C.

D. MUNCHBIN.EXE: The FORTRAN program MUNCHBIN.EXE was written to read binary data files for further file manipulation. It can perform the following data file manipulations:

- i) read binary files containing up to 8000 data points generated by SR245 or JY265 programs;
- ii) convert data point position into user units;
- iii) display partial or entire file content on screen;
- iv) print partial or entire file content;
- v) change signs of data points;
- vi) normalize data to the highest value in file;
- vii) write ASCII or binary files before or after file manipulation;
- viii) calculate values of the X axis according to the scan parameters, and write the ASCII file of the calculated X-axis.

One of the most useful features of the MUNCHBIN.EXE program is to transform the binary data files (as is or after manipulations) into ASCII files that can be used for other programs (e.g. Alligator Transforms FFT, Graphics programs). The reverse transformation (ASCII $\rightarrow$ binary files) was accomplished with another FORTRAN program, SPEWBIN.EXE, if further access to the JY265.EXE program became necessary (e.g. data reduction). The binary data files could be even further compressed using a public domain archiving program (PKWARE) for storage on floppy disks.

Table 4.6. List of Programs.

Program	Manufacturer	Function
DOS 3.0	IBM	Disk Operating System
FORTRAN 3.2	Microsoft	Compiler
FP II	Alligator Transforms	Transforms using Fast Fourier transform algorithm
SR265	Stanford Research Systems	Data acquisition and peripherals control
JY265	This work	Data acquisition and monochromator control
PROCOMM	Public Domain	Communication
PKX35A35	Public Domain	Archiving of data files
RESET	This work	Position of monochromator to a given wavenumber allowing for backlash
SID	This work	Scan identification, parameter calculations, hard copy of parameter list, and check list of manual readings
MUNCHBIN	This work	Binary data file manipulation (see sub-section 4.7.3.D)
SPEWBIN	This work	Reads ASCII data file and writes binary files to be used on SR265 or JY265

2 of/de 2

MicroD

4.8 OTHER EXPERIMENTAL CONDITIONS

This ends the automated part of the data acquisition. Other readings were written in a log book before each scan along with a hard copy of the scan parameters. The slit sizes of the monochromator were read from a vernier micrometer (1 unit = $0.5\ \mu\text{m}$) on the front panel of the Ramanor HG2. The time delay between the boxcar and the photomultiplier tube was measured and adjusted so that the Raman measurements were taken at the maximum of the laser pulse. The laser power measurements were read from a power meter (Photodyne 66XLA) fitted with a silicon detector head number 350.

4.9 TECHNICAL CONSTRAINTS

The difficulties associated with the long exposure to light of biological materials (e.g. thermal degradation photo-sensitivity and photo-oxidation)^{112,113} were also encountered during the recording of Raman spectra of Chl α . These obstacles and the ways used to circumvent them have been presented in this experimental section. In addition, there were problems more closely related to the nature of Chl a , namely: high fluorescence background, low scattering cross-section, and wavelength-dependent effects.^{28,80} The nature of these effects and their consequence on the recording of Raman spectra are presented below.

A. Fluorescence: The Q_y level of Chl α is very easily populated and has a high fluorescence quantum yield. The light emitted is so intense that it has precluded,

so far, resonance Raman study of the lower energy levels.^{6,11} (except for CARS and near-IR studies)¹²⁴

B. Low Scattering Cross-Section: Chl α shares several properties of porphyrins. One of them is a low resonance Raman cross-section, in spite of the presence of strong absorption bands.⁹²

C. Wavelength-Dependent Effects: The wavelength-dependent effects occur in a situation that requires the comparison of several Raman spectra over the range of an absorption band. It is important to distinguish two types of effects^{6,28,80}:

- i) absorption of incident photons, and
- ii) absorption of scattered photons.

The first effect can be controlled by either increasing the number of incident photons with a higher laser flux or increasing the number of absorbing molecules. A thin cell can also be used to ensure that the sample volume will remain the same over the range of excitation energy used. If no precautions are to be taken, the first effect will result in a decrease in the intensity of the Raman spectrum. Another way to prevent this is to use a solvent band located close to the Raman band to be monitored as an internal standard. Since the whole spectrum is affected the same way, the ratio of the Raman band of the molecule under study and the solvent band will remain the same no matter what the number of incident photons is.

When the incident photons are of higher energy than the absorption band, the energy of the scattered photons starts falling into the range of the absorption band. The net result is also a decrease in the intensity of the Raman spectrum, but this time it is highly dependent on the shift frequency. This becomes especially evident if the shifts are far apart and the absorption band is strong and narrow. In this circumstance, the spectrum must be corrected for re-absorption effect. This correction involves the ratio of the molar extinction coefficients corresponding to frequency of the scattered photon and the incident photon.

Consequently, three objectives had to be kept in mind during the preparation of Chl *a* samples:

- i) to increase the concentration in order to get a sufficient Raman signal;
- ii) to decrease the concentration in order to minimize the wavelength-dependent effects, and
- iii) to keep the concentration range within the limits in order to obtain the desired species (dimers in hexane, monomers in collidine) in the bulk of the solution.

The optimum concentrations to meet these requirements were approximately $5 \times 10^{-5} \text{M}$ in hexane and 10^{-4}M in collidine, in a 1 mm flowing cell. Solutions at these concentrations also demonstrated a linear relationship between the intensity of the Raman signal and the laser flux.

5. RESULTS AND DISCUSSION

5.1 INTRODUCTION

Raman spectroscopy is a powerful tool to study ground and lower excited electronic states. The recording of Raman spectra, however, requires attention to detail and cannot be achieved through routine techniques.

Although Chl *a* has strong absorbing bands, it is an inefficient scatterer. The intensities of Chl *a* Raman bands are 50 times smaller than the ones obtained from β -carotene.⁶ Chl *a* behaves in this respect like many other porphyrins.⁹² For that reason, Chl *a* has been studied at relatively high concentration (10^{-2} - 10^{-3} M) solution.

For this work, two types of Chl *a* species were studied; penta-coordinated monomers in collidine and dimers in hexane. The ratios of Chl *a* and solvent molecules were adjusted to provide optimum experimental conditions. That is to find a compromise between low concentrations to minimize wavelength-dependent effects and high concentration to maximize Raman signal. Excitation with back-scattering geometry was used to collect scattered photons efficiently and the use of a large volume of solution in a flowing cell helped to minimize photo-damage.

The results presented in this section have reached the following objectives:

- i) to implement and evaluate an automated data acquisition system for the

recording of nanosecond laser pulsed Raman spectroscopy system with a laboratory computer,

- ii) to observe resonance Raman spectra of monomeric Chl *a* in order to get insight on the building unit of the dimer (the solvent was chosen to mimic some of the properties of the other monomer);
- iii) to observe resonance Raman spectra of dimeric Chl *a* in order to get insight on the nature and the structure of bonding in Chl *a* dimers (the solvent was chosen to provide the desired degree of aggregation while keeping the solution in a concentration range suitable for the experiment);
- iv) to refine the knowledge of the electronic states of Chl *a* either as a monomer or a dimer with Raman excitation profiles, and
- v) to provide some initial work for theoretical Raman excitation profile calculations and/or predictions in order to extract excited state parameters that satisfy the experimental observations of the absorption spectrum and a Raman excitation profile (self-consistency).

5.2 DATA ACQUISITION

The resurgence of Raman spectroscopy in 1960s has generally been attributed to the development of reliable laser excitation sources. Coupled with the development of these powerful monochromatic sources has been the rapid development of photoelectric recording Raman instruments. During the same period, great strides have been made in the development of digital computers which are now omnipresent in laboratories. The prospect of coupling a photoelectric Raman instrument and a computer has become evident for some time.

- Part of the project for the work of this thesis was devoted to the implementation and evaluation of an automated data acquisition system. The need for such a system arises for the following reasons:

- i) noisy signals from the detection system owing to the nature of the pulsed excitation;
- ii) consecutive experiments that required consistency in experimental conditions;
- iii) simple and rapid data evaluation and reduction.

The primary purpose for coupling a computer to a Raman instrument is to collect data, so that rapid data evaluation can be prepared. In addition, a computer may also be used to control the course of the Raman experiment as long as the system provides a means to ensure that the computer and spectrometer are synchronized during data collection.

One significant advantage of using a computer to acquire data is the possibility of improving the S/N during collection. Two approaches were examined. One method consists of averaging by simply collecting the same spectrum a number of times and instructing the computer to average these spectra point-by-point. In this case, it is convenient to let the computer precisely reset the monochromator drive mechanism to the original starting frequency. The improvement achieved by this method is illustrated in Figure 5.1.

Figure 5.1

Improvement on the quality of Raman spectra as a result of averaging consecutive spectra. Spectra from a 10^{-5}M solution in hexane were used, the excitation frequency was $22\,331\text{ cm}^{-1}$ (447.8 nm). The x axis is the Raman shift in 1000 cm^{-1} , the upper spectrum represents the average of 5 scans, the lower spectrum is obtained after 1 scan only.

Figure 5.2

Improvement on the S/N of a Raman spectrum by averaging pulses fed to a A/D converter (averager). Spectra from a 10^{-5}M solution in hexane were used, the excitation frequency was $23\,540\text{ cm}^{-1}$ (424.8 nm) The x axis is the Raman shift in 1000 cm^{-1} , the upper spectrum is obtained with the average of 100 pulses/s, the lower spectrum is obtained with the averaging of 30 pulses/s.

Another method is to average pulses for a longer period of time for each point. The improvement in S/N is shown in Figure 5.2. This is particularly easy to implement on Raman instruments which use a stepping motor for the monochromator drive rather than a continuous scan motor.

5.3 DERIVATIVE SPECTROSCOPY

Derivative spectroscopy is a useful tool to reveal small or convolved bands in a spectrum. The absorption spectrum of Chl *a* in solution in a non-polar solvent demonstrates broader bands than in a polar solvent. This is the result of the aggregation of Chl *a* molecules. The 2nd derivatives of the absorption spectra of Chl *a* in hexane were calculated for several concentrations. Information on the electronic states hidden in the broad features have been extracted by the analysis of the 2nd derivative curve.

The 2nd derivatives was chosen over other order of derivation for the following reasons:

- i) the extremum positions of a 2nd derivative have similar positions to the original curve, and
- ii) a compromise is reached between improvement of resolution and deterioration of S/N.

The 2nd derivative data points were calculated from the original digitized absorption spectrum. Quantitative measurements of the 2nd derivative data were not carried out, however, the Soret and Q_y regions of the spectra were qualitatively analyzed.

Each region was reconstructed using Gaussian curves that fitted the observations obtained from the fundamental and 2nd derivative spectra. The results of the analysis of each portion of the spectrum will be discussed separately.

The absorption spectra of Chl *a* in collidine and hexane and their respective 2nd derivative curves were compared. They are representative of spectra of a monomeric and dimeric species in solution, respectively.

As the concentration of Chl *a* in hexane is increased, the broadening and the subsequent appearance of a shoulder to the red of the Q_y band indicates the presence of another band. Indeed, the 2nd derivative of the absorption spectra in this region have two minima (see Figure 5.3). These features have already been analyzed by Katz *et al.* in terms of splitting of the energy levels in the exciton formalism.¹³ In Table 5.1, the Q_y band and the 2nd derivative peak positions are listed for a range of concentrations spanning 4 orders of magnitude. Table 5.1 also lists the full-width at half-maximum (FWHM) of the absorption bands. The peak position and the FWHM of the Q_y band have traditionally been used to determine the presence of dimers in solutions. The examination of a curve containing overlapping bands by a 2nd derivative method makes a very sensitive test for the presence of multiple bands.

In the blue region (Soret), a small peak is located at 448 ± 1 nm ($22\,321\text{ cm}^{-1}$) which provides some evidence for an additional absorption band. Laser induced fluorescence and resonance Raman excitation profiles also show an enhancement at this wavelength.¹²⁵ No peak at 448 nm in the 2nd derivative spectra of any of the monomer solution (e.g. collidine, lutidine, ether, pyridine) could be detected.

Table 5.1 Absorption and 2nd derivative peak position for a Chl *a* solution in hexane 10^{-5} M.

Concentration (M)	Peak Position (nm)		FWHM
	Absorption		2 nd derivative
10^{-4}	665.8 ± 1	$678.1 \pm .7$ $662.3 \pm .7$	16 nm
10^{-5}	664.4 ± 1	$677.4 \pm .3$ $662.6 \pm .3$	16 nm
10^{-6}	664.8 ± 1	$676.6 \pm .6$ $662.7 \pm .6$	15 nm
10^{-7}	667.6 ± 1	$678.1 \pm .6$ $665.4 \pm .6$	12 nm
10^{-8}	669 ± 1	669 ± 1	10 nm

dimer

monomer

Figure 5.3

Absorption and 2nd derivative spectra of Chl *a* as a monomer (10^{-4} M in collidine) and as a dimer (10^{-5} M in hexane).

Figure 5.4

Attempt to fit the Soret and the Q_y region of the absorption spectrum Chl *a* in hexane solution. Two Gaussian curves are assumed to contribute to each band. The parameters of the Gaussian curves were chosen to fit the shape of both the absorption spectrum and the 2nd derivative.

An attempt was made to reconstruct each region of the absorption spectrum with two Gaussian curves. The parameters that would fit both the absorption spectrum and its second derivative could provide initial estimate for a more sophisticated deconvolution program. This analysis reveals that the best fit for the Q_y region is a ratio of the band 55:45 and of equal band-width taken as the one of the most diluted hexane solution (5.5 nm). For the blue band, a ratio of 5:1 and bandwidth of 7 and 5.5 nm were used. The results are illustrated in Figure 5.4

5.4 RESONANCE RAMAN STUDY OF Chl *a* IN COLLIDINE

The resonance Raman study includes the determination of the peak positions and of the excitation profiles of two bands; 1189 cm^{-1} and 1560 cm^{-1} . These Raman lines were chosen because they are representative of two modes of the macrocycle, C-N for the 1189 cm^{-1} shift and C_b-C_b for the 1560 cm^{-1} shift.

5.4.1 Resonance Raman Spectra

Solutions of Chl *a* in collidine contain mostly monomeric species as revealed by the absorption spectrum and its 2nd derivative as well as its fluorescence spectra. Several resonance Raman spectra were recording with a nanosecond pulsed laser excitation of a 10^{-4}M solution of Chl *a* in collidine. The excitation frequency range varied from $22\,180$ to $24\,815\text{ cm}^{-1}$ (450.8-403.0nm) which covered a large portion of the Soret absorption region of the chromophore. Typical resonance Raman spectra

Figure 5.5

Resonance Raman spectrum of Chl *a* in collidine (10^{-4} M). The excitation frequency is at $22\,929\text{ cm}^{-1}$ (436.1 nm). The rising background is caused by fluorescence from the Q_y band.

a)

b)

Figure 5.6

Raman excitation profiles of Chl *a* in collidine (10⁻⁴M) for two Raman shifts: a) 1189 cm⁻¹ b) 1555 cm⁻¹.

5.4.2 Raman Excitation Profiles

The Raman excitation profile (REP), reported in this work, is a plot of the intensities of a Raman intensities as a function of excitation frequency. The experimental intensities of two Raman bands of Chl *a* in solution have been monitored. The intensity of the monitored band from each spectrum (each spectrum has a different excitation frequency) has been corrected for wavelength-dependent effects and the ν^4 dependency in the following manner:

- i) taking the ratio of the band intensity, I , over the intensity of an internal standard band, I^0 , the 993 cm^{-1} band of collidine (breathing mode);
- ii) multiplying the intensity by $(\nu_L/\nu)^4$ where ν_L is the excitation frequency and ν the scattered photon frequency;
- iii) when necessary by correcting for reabsorption of the scattered photon by multiplying with (ϵ^0/ϵ) where ϵ^0 and ϵ are the molar extinction coefficients at the frequencies of the scattered photons from the solvent and the sample respectively.

5.4.3 Theoretical Calculations of Excitation Profiles

Theoretical calculations were performed to extract excited parameters from the REP of Chl *a* in solution. Two out of the three methods described in the theory section were used to evaluate these parameters:

- i) sum-over-states calculations were performed starting at the simplest level of approximations, and

- ii) a transform method was used to take into account all of the normal modes of vibrations involved in the excited electronic state.

These methods were chosen because they can provide a simple level of calculation and still provide information on the excited states. The time-dependent theory was left out in this work because it requires an accurate knowledge of the homogeneous line-width before the formalism can be developed. To deal with the multimode effect, that is certainly involved with REP of Chl *a*, the transform theory was applied. The two methods, sum-over-states and transform, therefore may complement each other. They may give indication on the relevancy of various assumptions since they are two independent ways of calculating a REP.

On one hand, the sum-over-states theory permit the calculation of the Raman intensities using a comprehensive approach since in the fully developed formalism all the normal modes of vibration have to be taken into account. On the other hand, the transform method analyzes the contribution of all the normal modes in the absorption spectrum and translates it into the corresponding scattering spectrum.

A. Sum-Over-States: The experimental data points were fitted to a line shape described by Equation 3.4.9 (Albrecht's A term). The excited-state parameter Γ_e was evaluated to 234 cm^{-1} and verified with the intensity of the absorption spectrum.

B. Transform Method: The experimental excitation profile of the 1189 cm^{-1} and the 1560 cm^{-1} are mapped over their respective calculated excitation profile in Figure 5. 6. Figure 5.7 is a superposition of the Q_y and the Soret band to show that

Figure 5.7

Comparison of the Q_y band in shape and position of the first vibronic transition with the Soret region.

Figure 5.8

Calculated excitation profile of the 1189 cm^{-1} band of Chl *a* in collidine by transform method from the digitized absorption of a) the Soret region and b) the Q_y region.

their first vibronic appears at the same relative position. The difference in intensity of the vibronic is taken as contribution by near-by η bands. The excitation profile for the 1189 cm^{-1} mode was calculated based on the respective absorption band region (see Figure 5.8).

5.5 RESONANCE RAMAN STUDY OF Chl *a* IN HEXANE

Other spectroscopic properties of this *in vitro* model have shown resemblance with spectroscopic evidences of *in vivo* systems. It seems that Chl *a* dissolved in hexane mimics the local environment of Chl in lipid layers of the plant and therefore represents an adequate model for the Chl interaction in this circumstance.

These experiment were designed to demonstrate the presence of at least two Chl *a* species co-existing in a dimeric solution. This was achieved by monitoring the intensities of two Raman bands with frequency shifts centered at 1655 cm^{-1} and 1705 cm^{-1} (resolution $\pm 6\text{ cm}^{-1}$) as a function of the excitation frequency. These frequency shifts have been assigned to the $\text{C}_9=\text{O}$ depending on whether they are "bound" (1655) or "free" (1705).

The 1555 cm^{-1} frequency shift was also monitored well into the 1-0 region of the Soret absorption band. Figure 5.9 shows that an enhancement at the vibronic position (409 nm).

Figure 5.9

Resonance excitation profile of Chl *a* (10⁻⁵M) in the 1-0 region, for the 1555 cm⁻¹.

Figure 5.10

Resonance excitation profile of Chl *a* (10⁻⁵ M) in the 1-0 region for the 1655 cm⁻¹.

Figure 5.11

Resonance excitation profile of Chl *a* (10⁻⁵ M) in the 1-0 region for the 1705 cm⁻¹.

C-O Region Raman Spectra

Chl a in Hexane 6500-65.6

105

Figure 5.12

Ratio of intensity of the the 1705 cm^{-1} band over the 1655 cm^{-1} in the 1-0 region

The $C_9=O$ bands are also enhanced in the vibronic position which confirms that they are in the plane of the conjugation path. However, the most interesting feature of this study is that the bound and free carbonyl are not enhanced to the same extent as a function of the excitation frequency. With an excitation at $22\,962\text{ cm}^{-1}$ (435.5 nm), the Raman shift at 1705 cm^{-1} (free) is preferably enhanced while at $22\,321\text{ cm}^{-1}$ (448.0 nm) the bound carbonyl band at 1655 cm^{-1} is enhanced and similarly, in the vibronic region. The individual enhancements are shown in Figure 5.10 and 5.11. The difference in enhancement is illustrated in Figure 5.12. These results are consistent with the 2 non-equivalent molecules linked to each other through the Mg atom and the $C_9=O$.

6. CONCLUSIONS

Data presented in this work have established several important points:

First, it is possible to acquire good quality spectra using pulsed excitation of Chl *a* in relatively dilute solution ($10^{-4} - 10^{-5}M$) at room temperature collecting the scattered photons with a back-scattering geometry. The quality of the spectrum can be further improved by digital signal averaging using a laboratory computer for data processing.

Second, high resolution Raman excitation profiles of Chl *a* in solution was obtained for the excitation range covering a good part of the Soret absorption region. It was shown that the excitation enhancement of the modes observed were governed by the Franck-Condon overlap factors (Albrecht's A term).

Third, monomeric and dimeric Chl *a* absorption spectra showed slight differences that can be revealed rapidly by derivative spectroscopy. A small band in dimeric solutions, located at 448 nm, could be resolved with this technique. It confirms previous assignment of this band obtained through laser-induced fluorescence and resonance Raman excitation profiles.

Fourth, the intensity of two Raman bands assigned to the free ($1705cm^{-1}$) and bound ($1655cm^{-1}$) $C_9=O$ carbonyl has been monitored. The difference in their respective excitation profile indicates the presence of at least two forms of Chl *a*

species co-existing in a solution that is composed mainly of dimers. Therefore, the Raman data are consistent with a T-shaped dimer model, although the data do not give any information on the angle between the two molecules which could be different from 90° .

Fifth, the theoretical resonance excitation profiles of Chl *a* as a monomer have been compared successfully to the experimental results obtained in the Soret region using standard assumptions (*e.g.* Franck-Condon overlap factor, no inhomogeneous broadening).

Sixth, a theoretical resonance excitation profile was derived for the Q_y , for which it is difficult to obtain resonance Raman spectroscopic data owing to the high fluorescence background in this region.

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Appendix A

Calculation of Chl *a* Content in a Flask

- 1) Concentration of Solution from Absorbance:

$$C \text{ (mol/L)} = \frac{\text{Absorbance}}{\epsilon \cdot l}$$

$$l = 1 \text{ cm and } \epsilon_{429} = 13\,500 \text{ L mol}^{-1}\text{cm}^{-1}$$

- 2) Number of Moles After Correction for Dilution Factor:

$$N' \text{ (mol)} = \frac{C \text{ (mol/L)} \cdot 25.00 \text{ ml} \cdot 10.0 \text{ ml} \cdot 10^{-3} \text{ L/ml}}{0.50 \text{ ml}}$$

- 3) Number of grams of Chl *a*:

$$N \text{ (g)} = N' \text{ (mol)} \cdot \text{M.W. (893.2 g/mol)}$$

Appendix B

Glossary of Terms

A/D	Analog to Digital conversion
ASCII	American Standard Code for Information Interchange. A 7 bit, 128 character code set which includes all upper and lower case alphabet, numerals, many common typewriter symbols and several control signs.
Async	Asynchronous communication protocol
Baud	Bits per second.
Binary	Base 2 number system whose digits are called bits.
Bit	Abbreviation of Binary Digit, the smallest unit of memory equal to 1 or 0.
Byte	A group of 8 bits treated as a whole with 256 possible combinations of one's and zero's, each combination representing a unique piece of information.
Compiler	Program which converts an entire high level language (e.g. FORTRAN) into machine code.
Disk	Mass storage device which encodes information magnetically on a spinning platter (disk).
Floppy disk	Small flexible disk usually holds less than 1 Mbyte.
FORTRAN	FORmula TRANslation, a high level compiler language particularly suited to calculation.
GPIB	General Purpose Interface Bus a digital data communications standard popular in instrumentation electronics.
Hardcopy	Permanent written or graphic record usually on paper
Hard disk	Disk which uses rigid platters. These platters can be spun quite rapidly to provide rapid access of mass storage devices. Hard disks can provide storage of several Mbytes of data. They are also called Winchester disks.

Interface	Collective word for hardware and software required for communication between a computer and its peripherals.
Mbytes	10^6 bytes.
Modem	Device used to connect devices together for communication over telephone lines. MOD ulation- DEM odulation.
Parallel	Simultaneous transfer of information over more than one line.
Port	Portion of a computer through which an interface to a device is made.
RAM	R andom A ccess M emory, memory which can be read and written into.
RS232	Standard for bit serial asynchronous data communication. The standard defines the format for data transmission, electrical specification for signal levels and mechanical dimensions of connectors.
Serial	Transfer of information 1 bit at a time
Slew	In motion control, the portion of a move made at a constant non-zero velocity.
Source code	Text of a program written in ASCII format. The compiler translates the source file into a machine code. After compiling, the machine code must be linked with the run-time library to produce an executable program.
TTL	T ransistor- T ransistor- L ogic, describes a common digital logic device family. TTL signals have two distinct states that are described with a voltage — a logical "zero" or "low" is represented by a voltage less than 0.8 V and a logical "one" or "high" is represented by a voltage from 2.5 to 5 V.
Volatile	Memory that is erased when power is turned off.

APPENDIX C

LISTINGS OF COMPUTER PROGRAMS

C.1 JYUSER.FOR

```
1 $STORAGE:2
2
3
4 C    TO BE USED WITH JOBIN YVON SPECTROMETER
5 C    SEPTEMBER 19, 1986
6
7 C          SUBROUTINE CALLED AT THE BEGINING OF ALL SCANS
8 C
9
10          SUBROUTINE BOSCNS
11
12          RETURN
13          END
14
15 C
16 C          SUBROUTINE CALLED AT THE END OF EACH SCAN
17 C
18          SUBROUTINE EOSCN
19
20 $INCLUDE:'SCNPAR.TXT'
    ***** Begin listing of: SCNPAR.TXT *****
1      COMMON/SCNPAR[near]/FACTOR(3),NOFBINS,KOFBINS,NBINS(3),KBINS(3),
2      1 NOFCHNS,KOFCHNS,FUNC(3),KFUNC(3),RATIO(3),KRATIO(3),NOFSCNS,
3      2 NSCN,XRLMIN(3),XRLMAX(3),YRLMIN(3),YRLMAX(3),XMIN(3),XMAX(3),
4      3 YMIN(3),YMAX(3),INPROG,PAUSED,START,SAVALL,SCL(3),KSCL(3),NPBIN,
5      4 NBIN,NRDY,SPCTR(3),KSPCTR(3),SINIT(3),KSI(3),
6      5 BLANK,TLINE,NAM4,SCLSTR,KCLSTR,TITLE,S
7 C
8          INTEGER*4 FACTOR,YMIN,YMAX
9          INTEGER*2 NRDY,FUNC,XMIN,XMAX
10         LOGICAL
INPROG,PAUSED,START,RATIO,KRATIO,SCL,KSCL,SAVALL
11         REAL*4 SPCTR,KSPCTR,KSI
12         CHARACTER BLANK*33,TLINE*33,SCLSTR(4,3),KCLSTR(4,3),NAM4*4
13         CHARACTER TITLE(8,3),S*1
    ***** End listing of:
SCNPAR.TXT
21 $INCLUDE:'ARRAYS.TXT'
    ***** Begin listing of: ARRAYS.TXT *****
1
2
```

```

3      COMMON/ARRAY1/CURPT(21030)
4      COMMON/ARRAY2/OLDPT(3,7010)
5      COMMON/ARRAY3/AVGPT(21030)
6
7      INTEGER*2 CURPT,OLDPT,AVGPT

```

***** End listing of: ARRAYS.TX

```

22
23 C    REWIND OF MONOCHROMATOR
24      CALL SND232 (T7;W0$)
25 C    SET PORT 8 = 5V TO INCREASE IN WAVENUMBERS
26      CALL SND232 ('S8=5$')
27 C    PULSE B2 (NBINS(NOFCHNS) * SFCTR(NOFCHNS) + 100 TIMES
28 C    TO BRING BACK TO START OF SCAN
29      MSTEP = (NBINS(NOFCHNS) * SFCTR(NOFCHNS) + 100) * 10
30      CALL PULSE (MSTEP, FALSE.)
31 C    SET PORT 8 = 0V TO DECREASE TO PROPER POSITION
32      CALL SND232 ('S8=0$')
33      CALL PULSE (800, FALSE.)
34      CALL PULSE (200, TRUE.)
35
36      RETURN
37      END

```

EOSCN Local Symbols

Name	Class	Type	Size	Offset
MSTEP	local	INTEGER*2	2	0000
AVGPT	ARRAY3	INTEGER*2	42060	0000
FACTOR	common	INTEGER*4	12	0000
NOFBIN	common	INTEGER*2	2	000c
KOFBIN	common	INTEGER*2	2	000e
NBINS	common	INTEGER*2	6	0010
KBINS	common	INTEGER*2	6	0016
NOFCHN	common	INTEGER*2	2	001c
KOFCHN	common	INTEGER*2	2	001e
FUNC	common	INTEGER*2	6	0020
KFUNC	common	INTEGER*2	6	0026
RATIO	common	LOGICAL*2	6	002c
KRATIO	common	LOGICAL*2	6	0032
NOFSCN	common	INTEGER*2	2	0038
NSCN	common	INTEGER*2	2	003a
XRLMIN	common	REAL*4	12	003c
XRLMAX	common	REAL*4	12	0048
YRLMIN	common	REAL*4	12	0054
YRLMAX	common	REAL*4	12	0060
XMIN	common	INTEGER*2	6	006c
XMAX	common	INTEGER*2	6	0072
YMIN	common	INTEGER*4	12	0078
YMAX	common	INTEGER*4	12	0084

```

INPROG.....common LOGICAL*2      2  0090
PAUSED.....common LOGICAL*2      2  0092
START.....common LOGICAL*2      2  0094
SAVALL.....common LOGICAL*2      2  0096
SCL.....common LOGICAL*2      6  0098
KSCL.....common LOGICAL*2      6  009e
NPBIN.....common INTEGER*2      2  00a4
NBIN.....common INTEGER*2      2  00a6
NRDY.....common INTEGER*2      2  00a8
SFCTR.....common REAL*4      12  00aa
KSFCTR.....common REAL*4      12  00b6
SINT.....common REAL*4      12  00c2
KSI.....common REAL*4      12  00ce
BLANK.....common      33  00da
TLINE.....common      33  00fb
NAM4.....common      4  011c
SCLSTR.....common      12  0120
KCLSTR.....common      12  012c
TITLE.....common      24  0138
S.....common      1  0150
CURPT.....ARRAY1 INTEGER*2      42060  0000
OLDPT.....ARRAY2 INTEGER*2      42060  0000

```

38

39 C

40 C SUBROUTINE CALLED AT EACH POINT IN SCAN

41 C

42 SUBROUTINE EACHPT

43

44 \$INCLUDE:'SCNPAR.TXT'

T

46

47 C MOTOR COMMANDS

48 C 10 TTL PULSES FOR 1 cm-1

49

50 CALL SND232 (T7;W0\$)

51 C SET B1 AT 0 TTL TO DECREASE IN WAVENUMBERS

52 CALL SND232 ('S8=0\$')

53 C PULSES B2 MSTEP TIMES

54 MSTEP = sfctr(nofchns) * 10

55 CALL PULSE (MSTEP,.FALSE.)

56

57 RETURN

58 END

EACHPT Local Symbols

Name	Class	Type	Size	Offset
MSTEP.....	local	INTEGER*2	2	0002
FACTOR.....	common	INTEGER*4	12	0000
NOFBIN.....	common	INTEGER*2	2	000c

KOFBIN.....	common	INTEGER*2	2	000e
NBINS.....	common	INTEGER*2	6	0010
KBINS.....	common	INTEGER*2	6	0016
NOFCHN.....	common	INTEGER*2	2	001c
KOFCHN.....	common	INTEGER*2	2	001e
FUNC.....	common	INTEGER*2	6	0020
KFUNC.....	common	INTEGER*2	6	0026
RATIO.....	common	LOGICAL*2	6	002c
KRATIO.....	common	LOGICAL*2	6	0032
NOFSCN.....	common	INTEGER*2	2	0038
NSCN.....	common	INTEGER*2	2	003a
XRLMIN.....	common	REAL*4	12	003c
XRLMAX.....	common	REAL*4	12	0048
YRLMIN.....	common	REAL*4	12	0054
YRLMAX.....	common	REAL*4	12	0060
XMIN.....	common	INTEGER*2	6	006c
XMAX.....	common	INTEGER*2	6	0072
YMIN.....	common	INTEGER*4	12	0078
YMAX.....	common	INTEGER*4	12	0084
INPROG.....	common	LOGICAL*2	2	0090
PAUSED.....	common	LOGICAL*2	2	0092
START.....	common	LOGICAL*2	2	0094
SAVALL.....	common	LOGICAL*2	2	0096
SCL.....	common	LOGICAL*2	6	0098
KSCL.....	common	LOGICAL*2	6	009e
NPBIN.....	common	INTEGER*2	2	00a4
NBIN.....	common	INTEGER*2	2	00a6
NRDY.....	common	INTEGER*2	2	00a8
SFCTR.....	common	REAL*4	12	00aa
KSFCTR.....	common	REAL*4	12	00b6
SINT.....	common	REAL*4	12	00c2
KSI.....	common	REAL*4	12	00ce
BLANK.....	common		33	00da
TLINE.....	common		33	00fb
NAM4.....	common		4	011c
SCLSTR.....	common		12	0120
KCLSTR.....	common		12	012c
TITLE.....	common		24	0138
S.....	common		1	0150
CURPT.....	ARRAY1	INTEGER*2	42060	0000
OLDPT.....	ARRAY2	INTEGER*2	42060	0000
AVGPT.....	ARRAY3	INTEGER*2	42060	0000

59

60

61 C TO ACTIVATE STEPPER MOTOR

62

63 SUBROUTINE PULSES (MSTEP,SLOW)

64

65 LOGICAL SLOW

66

67 C PULSES B2 MSTEP TIMES TO ACTIVATE STEPPER MOTOR

```

68 C
69 C      DELAY WHEN MOTOR HAS TO BE SLOW
70          IF (SLOW) THEN
71              DO 35 K=1,5000
72 35          CONTINUE
73              ENDIF
74 40      CALL SND232 (SB2=0;W3;SB2=1;W3$)
75          RETURN
76          END

```

PULSE Local Symbols

Name	Class	Type	Size	Offset
SLOW.....	param			0006
MSTEP.....	param			000a
J.....	local	INTEGER*2	2	0004
K.....	local	INTEGER*2	2	0006

Global Symbols

Name	Class	Type	Size	Offset
ARRAY1.....	common		42060	0000
ARRAY2.....	common		42060	0000
ARRAY3.....	common		42060	0000
BOSCNS.....	FSUBRT	***	***	0000
EACHPT.....	FSUBRT	***	***	00c7
EOSCN.....	FSUBRT	***	***	0005
PULSE.....	FSUBRT	***	***	0130
SCNPAR.....	common		337	0000
SND232.....	extern	***	***	***

C.2 MUNCHBIN.FOR

C.2.1 BR2.FOR

```

1
2      PROGRAM BR2
3
4      $INCLUDE:'FILEB.TXT'
      ***** Begin listing of: FILEB.TXT
1      $LARGE
2          COMMON/FILEB[far]/Y,Yy,MAX,MIN,DEP,FIN,
3      + NBINS,NCHNS,IDEP,IFIN,FACTOR,XBIN,NBIN,OLDPT,
4      + ACT,ANS,UNITS,UNITY,
5      + RAWDAT,YESCL,RATIO,ESC,
6      + SCALE,SINIT
7

```

```

8      REAL*8      Y(3,4100),Yy(1,4100),MAX,MIN
9      INTEGER*4    NBINS,NCHNS,IDEF,IFIN,FACTOR,XBIN,NBIN
10     INTEGER*2    OLDPT(3,4100)
11     LOGICAL*4    RAWDAT,YESCL,RATIO,ESC
12     CHARACTER    UNITS*4,ACT*3,ANS*3,UNITY*4
13
14 C      FILEB.TXT IS A FILE OF COMMON VARIABLES
15
16 ***** End listing of: FILEB.TXT
17
18 CHARACTER      DNAME*12
19
20 1      write (*,*)
21
22 NS = 0
23 NN = 0
24 FLAG = 1.
25 WRITE (*,100)
26 C      READ NAME OF DATA FILE TO READ
27 CALL GETD(DNAME)
28 C      OPEN UNIT TO READ BINARY FILE
29 OPEN (12,FILE=DNAME,STATUS='OLD',FORM='BINARY',ERR=2000)
30 FLAG = 0.
31 READ (12) RAWDAT
32 IF (RAWDAT) THEN
33 READ (12) NCHNS,NBINS,
34 +      ((OLDPT(I,J),I=1,NCHNS),J=1,NBINS),
35 +      YESCL,SCALE,SINT,UNITS
36 ELSE
37 NCHNS = 1
38 READ (12) NBINS,RATIO,FACTOR,XRLMIN,XRLMAX,YRLMIN,YRLMAX,
39 +      YESCL,SCALE,SINT,UNITS,
40 +      ((OLDPT(I,J),I=1,NCHNS),J=1,NBINS)
41 ENDIF
42
43 C      INTEGER ARRAY OF DATA IS PUT INTO A DOUBLE PRECISION REAL FILE
44 DO 2 J = 1,NBINS
45 DO 3 I = 1,NCHNS
46 Y(I,J) = DBLE(REAL(OLDPT(I,J))/400.)
47 3      CONTINUE
48 2      CONTINUE
49
50 C      THIS ALLOWS TO CHOOSE THE START AND END OF DISPLAYED OR
51 C      PRINTED FILES.
52 DO 4 I = 1,NCHNS
53 UNITY = UNITS
54 XBIN = 1
55 IDEP = 1
56 IFIN = NBINS
57 1000 WRITE (*,105)
58 IF (NS.EQ.1) WRITE (*,300)
59 IF (NN.EQ.1) THEN

```

```

48      WRITE (*,310)
49      ELSE
50      WRITE (*,320)
51      ENDIF
52      WRITE (*,400)
53      WRITE (*,106) \
54      READ (*,'(A)') ACT
55      WRITE (*,500) ACT
56
57 C      CHOICE OF DIFFERENT ACTIONS TO BE DONE ON FILE
58 C      D:DISPLAY P:PRINT N:NORMALIZE O:REMEMBERS ORIGINAL DATA
59 C      AFTER NORMALIZATION W: WRITE A NEW BINARY OR ASCII FILE
60 C      A:ACCESS TO ANOTHER FILE S:CHANGE SIGN IN FRONT OF Y AXIS
61 C      X:EXIT PROGRAM U:TRANSFORM VALUE IN UNITS FROM FILE IN BIN
62 C      NUMBER V:CHANGE BEGINNING,END AND INTERVAL OF FILE
63
64      IF (ACT.EQ.'V'.OR.ACT.EQ.'v') CALL CLIP (1)
65      IF (ACT.EQ.'D'.OR.ACT.EQ.'d') CALL COPIE (1)
66      IF (ACT.EQ.'P'.OR.ACT.EQ.'p') CALL COPIE (2)
67      IF (ACT.EQ.'A'.OR.ACT.EQ.'a') GOTO 1
68      IF (ACT.EQ.'U'.OR.ACT.EQ.'u') CALL BINUM
69      IF (ACT.EQ.'N'.OR.ACT.EQ.'n') THEN
70          CALL NORMAL (1,J)
71          NN = 1
72      ENDIF
73      IF (ACT.EQ.'O'.OR.ACT.EQ.'o') THEN
74          CALL NORMAL (2,J)
75          NN = 0
76      ENDIF
77      IF (ACT.EQ.'W'.OR.ACT.EQ.'w') THEN
78          WRITE (*,110)
79          READ (*,'(A)') ACT
80          WRITE (*,500) ACT
81          IF (ACT.EQ.'A'.OR.ACT.EQ.'a') CALL COPIE (3)
82          IF (ACT.EQ.'B'.OR.ACT.EQ.'b') then
83              write (*,*) '*****'
84          CALL COPIE (3)
85          endif
86          IF (ACT.EQ.'C'.OR.ACT.EQ.'c') CALL COPIE (6)
87          WRITE (*,400)
88          ENDIF
89      IF (ACT.EQ.'S'.OR.ACT.EQ.'s') THEN
90          DO 7 J = 1,NBINS
91              Y(I,J) = -Y(I,J)
92 7      CONTINUE
93          WRITE (*,300)
94          WRITE (*,400)
95          NS = 1
96          ENDIF
97      IF (ACT.EQ.'X'.OR.ACT.EQ.'x') GOTO 1001
98
99      GOTO 1000

```

```

100 4    CONTINUE
101
102 2000  IF (FLAG.EQ.1.) THEN
103      WRITE (*,200) DNAME
104      GOTO 1
105      ENDIF
106
107 100    FORMAT (/ ' THIS PROGRAM WILL READ UP TO 8000 DATA POINTS'/
108      +      ' FROM A BINARY FILE'/)
109 105    FORMAT (/10X,'TO CHANGE UNITS VALUES IN BIN NO. ENTER -> U'/
110      + 10X,'TO PRINT OUT ENTER -> P'/
111      + 10X,'TO DISPLAY ON THE SCREEN ENTER -> D'/
112      + 10X,'TO NORMALIZE DATA ENTER -> N'/
113      + 10X,'TO GET BACK TO ORIGINAL DATA ENTER -> O'/
114      + 10X,'TO CHANGE SIGN ON THE Y AXIS ENTER -> S'/
115      + 10X,'TO CHANGE START AND END VALUES ENTER -> V'/
116      + 10X,'TO WRITE A NEW DATA FILE ENTER -> W'/
117      + 10X,'TO ACT ON ANOTHER DATA FILE ENTER -> A'/
118      + 10X,'TO EXIT PROGRAM ENTER -> X'/)
119 106    FORMAT (/10X,'ENTER -> P,D,N,O,S,V,W,A, or X TO CONTINUE--> \')
120 110    FORMAT (/10X,'TO WRITE AN ASCII FILE (Y ONLY) ENTER -> A'/
121      + 10X,' ( phase plane deconvolution) '/
122      + 10X,'TO WRITE A BINARY FILE ENTER -> B'/
123      + 10X,' ( SR265 subroutines) '/
124      + 10X,'TO WRITE AN ASCII FILE (X & Y) ENTER -> C'/
125      + 10X,' ( plots with ColorPro and GRA-FIT) '//10X,
126      + 'ENTER -> A,B, or C --> \')
127 200    FORMAT (/5X,A11,' IS NOT A BINARY FILE'/
128      + 12X,' --TRY AGAIN-- '/')
129 210    FORMAT (/10X,'WORKING ON : ',A11)
130 300    FORMAT (/10X,'SIGN CHANGED')
131 310    FORMAT (/10X,'NORMALIZED')
132 320    FORMAT (/10X,'NOT NORMALIZED')
133 400    FORMAT (/)
134 500    FORMAT (/10X,'SELECTION: ',A1//)
135
136 1001    CLOSE(12)
137 1002    CONTINUE
138
139      END

```

main Local Symbols

Name	Class Type	Size	Offset
I	local INTEGER*4	4	0012
J	local INTEGER*4	4	0016
XRLMIN	local REAL*4	4	001a
FLAG	local REAL*4	4	001c
YRLMIN	local REAL*4	4	0022
XRLMAX	local REAL*4	4	0026

NN.....	local INTEGER*4	4	002a
YRLMAX.....	local REAL*4	4	002e
NS.....	local INTEGER*4	4	0032
DNAME.....	local CHAR*12	12	0036
Y.....	FILEB REAL*8	98400	0000
YY.....	FILEB REAL*8	32800	8060
MAX.....	FILEB REAL*8	8	0080
MIN.....	FILEB REAL*8	8	0088
DEP.....	FILEB REAL*4	4	0090
FIN.....	FILEB REAL*4	4	0094
NBINS.....	FILEB INTEGER*4	4	0098
NCHNS.....	FILEB INTEGER*4	4	009c
IDEP.....	FILEB INTEGER*4	4	00a0
IFIN.....	FILEB INTEGER*4	4	00a4
FACTOR.....	FILEB INTEGER*4	4	00a8
XBIN.....	FILEB INTEGER*4	4	00ac
NBIN.....	FILEB INTEGER*4	4	00b0
OLDPT.....	FILEB INTEGER*2	24600	00b4
ACT.....	FILEB	3	60cc
ANS.....	FILEB	3	60cf
UNITS.....	FILEB	4	60d2
UNITY.....	FILEB	4	60d6
RAWDAT.....	FILEB LOGICAL*4	4	60da
YESCL.....	FILEB LOGICAL*4	4	60de
RATIO.....	FILEB LOGICAL*4	4	60e2
ESC.....	FILEB LOGICAL*4	4	60e6
SCALE.....	FILEB REAL*4	4	60ea
SINIT.....	FILEB REAL*4	4	60ee

Global Symbols

Name	Class	Type	Size	Offset
BINUM.....	extern	***	***	***
CLIP.....	extern	***	***	***
COPIE.....	extern	***	***	***
FILEB.....	common		155890	0000
GETD.....	extern	***	***	***
NORMAL.....	extern	***	***	***
main.....	FSUBRT	***	***	0000

C2.2 BCLIP.FOR

```

1      SUBROUTINE CLIP(I)
2
3      $INCLUDE:FILEB.TXT
4
5      WRITE (*,100) IDEP,IFIN,NBINS
6 1000   WRITE (*,*) ' BEGINNING'
7      READ (*,*) IDEP

```

```

8      IF (IDEP.LT.1) THEN
9      WRITE (*,*) ' MINIMUM VALUE FOR START OF SCAN IS 1'
10     GOTO 1000
11     ENDIF
12
13 1200  WRITE (*,*) ' END'
14      READ (*,*) IFIN
15      IF (IFIN.GT.NBINS) THEN
16      WRITE (*,*) ' MAXIMUM VALUE FOR END OF SCAN IS'
17      WRITE (*, '(10X,I4)') NBINS
18      GOTO 1200
19      ENDIF
20      IF (IFIN.LT.2) THEN
21      WRITE (*,*) ' MINIMUM VALUE FOR END OF SCAN IS 2'
22      GOTO 1200
23      ENDIF
24
25 1400  WRITE (*,*) ' INTEGER INTERVAL BETWEEN POINTS'
26      READ (*,*) XBIN
27      IF (XBIN.LT.1) THEN
28      WRITE (*,*) ' MINIMUM VALUE FOR INTERVAL IS 1'
29      GOTO 1400
30      ENDIF
31
32      WRITE (*,101) IDEP,I,IFIN,XBIN
33      READ (*, '(A1)') ACT
34      IF (ACT.EQ.'V'.OR.ACT.EQ.'v') GOTO 1000
35
36 100   FORMAT (/10X,' TO ACT ON PART OF A FILE ONLY'/
37      + ' ENTER -> V TO CHANGE THESE VALUES'/
38      + '          |      '//
39      + '          |      '//
40      + '          v      v//
41      + 16X,I4,5X,I4//
42      + ' ORIGINAL VALUES 1  ',I4,/)
43 101   FORMAT (10X,' THE FIRST POINT:',I4,' '//
44      + 31X,' > OF CHANNEL ',I1/
45      + 10X,' THE LAST POINT:',I4,' '//
46      + 10X,' WITH INTERVAL OF ',I4,' BETWEEN POINTS'//
47      + 15X,' PRESS ENTER KEY /25X,'OR'/15X,
48      + ' TO CHANGE VALUES ENTER V -> \)
49
50      RETURN
51      END

```

CLIP Local Symbols

Name	Class	Type	Size	Offset
I	param		0006	
Y	FILEB	REAL*8	98400	0000

YY.....	FILEB	REAL*8	32800	8060
MAX.....	FILEB	REAL*8	8	0080
MIN.....	FILEB	REAL*8	8	0088
DEP.....	FILEB	REAL*4	4	0090
FIN.....	FILEB	REAL*4	4	0094
NBINS.....	FILEB	INTEGER*4	4	0098
NCHNS.....	FILEB	INTEGER*4	4	009c
IDEP.....	FILEB	INTEGER*4	4	00a0
IFIN.....	FILEB	INTEGER*4	4	00a4
FACTOR.....	FILEB	INTEGER*4	4	00a8
XBIN.....	FILEB	INTEGER*4	4	00ac
NBIN.....	FILEB	INTEGER*4	4	00b0
OLDPT.....	FILEB	INTEGER*2	24600	00b4
ACT.....	FILEB		3	60cc
ANS.....	FILEB		3	60cf
UNITS.....	FILEB		4	60d2
UNITY.....	FILEB		4	60d6
RAWDAT.....	FILEB	LOGICAL*4	4	60da
YESCL.....	FILEB	LOGICAL*4	4	60de
RATIO.....	FILEB	LOGICAL*4	4	60e2
ESC.....	FILEB	LOGICAL*4	4	60e6
SCALE.....	FILEB	REAL*4	4	60ea
SINT.....	FILEB	REAL*4	4	60ec

8

Global Symbols

Name	Class	Type	Size	Offset
CLIP.....	FSUBRT	***	***	0000
FILEB.....	common		155890	0000

C.23 BCOPIE.FOR

```

1
2 C   DISPLAY, PRINT, WRITE TO ASCII OR BINARY FILE
3 C   AT DEFAULT OR GIVEN VALUES.
4     SUBROUTINE COPIE(NUM)
5
6     INCLUDE 'FILEB.TXT'
7
8     INTEGER*4    LDIF
9     CHARACTER    RNAME*10,RAMANAME*4,RDAT*5
10    CHARACTER    TEXT*25
11    DIMENSION    RDAT(3),RNAME(3),X(8000)
12
13    OPEN (2,FILE='PRN')
14
15    IF (RAWDAT) THEN
16    TEXT = 'IS A RAW DATA FILE'
```



```

17      ELSE
18      TEXT = 'IS NOT A RAW DATA FILE'
19      RAWDAT = .TRUE.
20      ENDIF
21
22      SCALING = REAL(XBIN)*SCALE
23      CALL NORMAL (3,I)
24
25 C      DISPLAY FILE STARTING AT I TO NBINS OR IDEP TO IFIN
26      IF (NUM.EQ.1) THEN
27      WRITE (*,300) DNAME,TEXT
28          DO 11 I=1,NCHNS
29      WRITE (*,400) I,NBINS
30      WRITE (*,450) IDEP,IFIN
31      WRITE (*,451) SCALING,UNITY
32      WRITE (*,500) UNITY
33          DO 15 J=IDEP,IFIN,XBIN
34      WRITE (*,600) J,SNGL(Y(I,J))
35      IF (J.EQ.(IDEP+15*XBIN)) THEN
36      PAUSE
37      IF (J.LT.IFIN) WRITE (*,*) ' MORE TO COME'
38      ENDIF
39      IF (IFIN.LT.(IDEP+15*XBIN).AND.J.EQ.IFIN) PAUSE
40 15      CONTINUE
41      WRITE (*,700)
42 11      CONTINUE
43      RETURN
44      ENDIF
45
46 C      PRINT FILE STARTING AT DEFAULT OR GIVEN IDEP AND IFIN
47      IF (NUM.EQ.2) THEN
48      WRITE (2,300) DNAME,TEXT
49          DO 2 I=1,NCHNS
50      WRITE (*,400) I,NBINS
51      WRITE (2,450) IDEP,IFIN
52      WRITE (2,451) SCALING,UNITY
53          DO 220 J=IDEP,IFIN,XBIN
54      WRITE (2,600) J,SNGL(Y(I,J))
55 220      CONTINUE
56      WRITE (2,700)
57 2      CONTINUE
58      RETURN
59      ENDIF
60
61      WRITE (*,31)
62          IF (NCHNS.EQ.1) THEN
63      RDAT(1) = '.DAT0'
64      ELSE
65      WRITE (*,32)
66          DO 100 I=1,NCHNS
67      RDAT(I)(1:2)=CHAR(I+48)
68      RDAT(I)(2:2+4)='.DAT'

```

```

69 100      CONTINUE
70          ENDIF
71
72          WRITE (*,33)
73          READ (*,'(A4)') RAMANAME
74
75          DO 110 I=1,NCHNS
76              RNAME(I)(1:4)=RAMANAME
77              RNAME(I)(5:5+6)=RDAT(I)
78 110      CONTINUE
79
80          IF (NUM.EQ.3) THEN
81              DO 30 I=1,NCHNS
82                  OPEN ((I+2),FILE=RNAME(I),STATUS='NEW',FORM='BINARY')
83
84  C          WRITE BINARY FILE FROM IDEP TO IFIN
85                  write (*,*) ' '
86                  write (*,*) '      Writing a binary file '
87                  write (*,*) ' '
88                  FACTOR = 1
89                  NBIN = INT(((IFIN - IDEP)/XBIN)+1)
90                  START = REAL(IDEP) * SCALING + SINIT
91                  DO 3 J=IDEP,IFIN,XBIN
92                      OLDPT(I,J)=IDINT(Y(I,J)*400.)
93 3          CONTINUE
94                  WRITE (I+2) RAWDAT,FACTOR,NBIN
95                  DO 39 J=IDEP,IFIN,XBIN
96                      WRITE (I+2) OLDPT(I,J)
97 39      CONTINUE
98                  WRITE (I+2) YESCL,SCALING,START,UNITS
99
100             CLOSE ((I+2),STATUS='KEEP')
101             WRITE (*,250) RNAME(I)
102 30      CONTINUE
103
104          RETURN
105          ENDIF
106
107  C          WRITE ASCII FILE ( X & Y )
108              IF (NUM.EQ.6) THEN
109
110                  DO 60 I=1,NCHNS
111  C          FIND MIN AND MAX VALUES FOR EACH CHANNEL
112              CALL NORMAL (3,I)
113                  write (*,*) ' '
114                  write (*,*) '      Writing a file '
115                  write (*,*) ' '
116              OPEN ((I+5),FILE=RNAME(I),STATUS='NEW')
117
118              WRITE (*,'(A\)' ) START OF SCAN      ->
119              READ (*,*) X0
120              WRITE (*,'(A\)' ) END OF SCAN          ->

```

```

121 READ (*,*) DFINL
122 WRITE (*,*) ' WAIT PLEASE'
123
124 LDIF = IFIN - IDEP
125 RES= ABS(DFINL - X0)/REAL(LDIF)
126 IF (DFINL.LT.X0) X0 = -X0
127 IF (LDIF.GT.2000) THEN
128   IF (XBIN.LT.(INT(LDIF/2000+1))) XBIN = INT(LDIF/2000+1)
129   ENDF
130 WRITE (*,627) NBINS,XBIN
131 DO 63 J=IDEP,IFIN,XBIN
132   WRITE (*,(A)) ' IS THAT ALRIGHT?      Y/N  -> '
133   READ (*,(A1)) ACT
134   WRITE (*,*) ' CHOOSE THE APPROPRIATE INTERVAL AGAIN '
135   IF (ACT.EQ.'N'.OR. ACT.EQ.'n') CALL CLIP(I)
136 63  X(J) = ABS(X0 + REAL(J-IDEP)*RES)
137
138   WRITE (*,(A)) ' TIME SCAN? Y/N      -> '
139   READ (*,(A1)) ACT
140   IF (ACT.EQ.'Y'.OR. ACT.EQ.'y') GOTO 1000
141
142   WRITE (*,(A)) ' WAVELENGTH IN nm OR cm-1? n/c -> '
143   READ (*,(A1)) ACT
144   IF (ACT.EQ.'N'.OR. ACT.EQ.'n') THEN
145     DO 65 J=IDEP,IFIN,XBIN
146 65  X(J) = 10E6/X(J)
147     ENDF
148
149   WRITE (*,*) ' FLUORESCENCE OR RAMAN SHIFT PLOT?'
150   WRITE (*,(A)) '      F/R      -> '
151   READ (*,(A1)) ANS
152   IF (ANS.EQ.'R'.OR. ANS.EQ.'r') THEN
153     WRITE (*,(A)) ' EXCITATION WAVELENGTH IN nm -> '
154     READ (*,*) WEXC
155     EXCW = 10E6/WEXC
156     WRITE (*,610) WEXC,EXCW
157     WRITE (*,*) ' JUST WAIT PLEASE'
158     IF (ACT.EQ.'N'.OR. ACT.EQ.'n') EXCW = WEXC
159     DO 67 J =IDEP,IFIN,XBIN
160     X(J) = EXCW - X(J)
161 67  CONTINUE
162     WRITE (*,*) ' RAMAN SHIFT FIRST AND LAST POINTS'
163     ELSE
164     WRITE (*,*) ' FLUORESCENCE FIRST AND LAST POINTS'
165     ENDF
166
167
168   WRITE (*,615)
169   WRITE (*,620) (X(J),Y(I,J),J=1,(IDEP+XBIN))
170   WRITE (*,700)
171   WRITE (*,620) (X(J),Y(I,J),J=(IFIN-XBIN),IFIN)
172   WRITE (*,700)

```

```

173      WRITE (*,*) ' WAIT PLEASE'
174
175 1000      DO 6 J=IDEP,IFIN,XBIN
176          OLDPT(I,J)=IDINT(Y(I,J)*400.)
177 6        CONTINUE
178      WRITE (*,625)
179          DO 69 J=IDEP,IFIN,XBIN
180      WRITE ((I+5),620) X(J),SNGL(Y(I,J))
181 69      CONTINUE
182      WRITE ((I+5),800)
183      WRITE (*,250) RNAME(I)
184
185      WRITE (*,(A)) ' PRINT OUT OF INFORMATION? Y/N -> '
186      READ (*,(A1)) ANS
187      IF (ANS.EQ.'Y'.OR. ANS.EQ.'y') THEN
188          WRITE (2,605)
189          IF (I.GT.1) THEN
190              WRITE (2,631) RNAME(I)
191          ELSE
192              WRITE (2,630) RNAME(I)
193          ENDIF
194      IF (ACT.EQ.'N'.OR. ACT.EQ.'n')
195  +      WRITE (2,610) WEXC,EXCW
196          WRITE (2,640) ABS(X0),DFINL
197          WRITE (2,650) X(IDEP),X(IFIN)
198          WRITE (2,660) MIN,MAX
199          WRITE (2,605)
200          WRITE (2,700)
201          WRITE (2,700)
202      ELSE
203          WRITE (*,605)
204          IF (I.GT.1) THEN
205              WRITE (*,631) RNAME(I)
206          ELSE
207              WRITE (*,630) RNAME(I)
208          ENDIF
209      IF (ACT.EQ.'N'.OR. ACT.EQ.'n')
210  +      WRITE (*,610) WEXC,EXCW
211          WRITE (*,640) ABS(X0),DFINL
212          WRITE (*,650) X(IDEP),X(IFIN)
213          WRITE (*,660) MIN,MAX
214          WRITE (*,605)
215          WRITE (*,700)
216      ENDIF
217
218      CLOSE ((I+5),STATUS='KEEP')
219 60      CONTINUE
220
221      RETURN
222      ENDIF
223
224 C      WRITE ASCII FILE (Y ONLY)

```

```

225         IF (NUM.EQ.9) THEN
226             DO 90 I=1,NCHNS
227                 OPEN ((I+8),FILE=RNAME(I),STATUS='NEW')
228
229 C         WRITE ASCII FILE FROM IDEP TO IFIN
230         WRITE (*,*) ' IS IT FOR COLOR-PRO?'
231         READ (*, '(A)') ANS
232         IF (ANS.EQ.'Y'.OR.ANS.EQ.'y') THEN
233             LDIF = IFIN - IDEP
234             XBIN = INT(LDIF/240.)+1
235             WRITE (*,627) NBINS,XBIN
236         ENDIF
237         WRITE (*,*) 'DO YOU NEED XDATA FILE?'
238         READ (*, '(A)') ANS
239         IF (ANS.EQ.'Y'.OR.ANS.EQ.'y') THEN
240             OPEN (1,FILE='XDAT.DAT',STATUS='NEW')
241             WRITE (*, '(A)') ' START OF SCAN'      ->
242             READ (*,*) X0
243             WRITE (*, '(A)') ' END OF SCAN'        ->
244             READ (*,*) DFINL
245             RES= ABS(DFINL - X0)/REAL(LDIF)
246             IF (DFINL.LT.X0) X0 = -X0
247             DO 79 J=IDEP,IFIN,XBIN
248                 X(J) = ABS(X0 + REAL(J-IDEP)*RES)
249                 WRITE (1,*)X(J)
250 79         CONTINUE
251             CLOSE (1,STATUS='KEEP')
252         ENDIF
253             DO 9 J=IDEP,IFIN,XBIN
254                 OLDPT(I,J)=IDINT(Y(I,J)*400.)
255 9         CONTINUE
256         WRITE (*,625)
257         vft = 0.0
258         WRITE (*,*) 'IS IT FOR FFT ?'
259         READ (*, '(A)') ANS
260         IF (ANS.EQ.'Y'.OR.ANS.EQ.'y') THEN
261             DO 19 J=IDEP,IFIN,XBIN
262                 WRITE ((I+8),918) SNGL(Y(I,J)),vft
263 19         CONTINUE
264         ELSE
265             DO 59 J=IDEP,IFIN,XBIN
266                 WRITE ((I+8), '(5X,F10.3)') SNGL(Y(I,J))
267 59         CONTINUE
268         WRITE (*,250) RNAME(I)
269         ENDIF
270
271         CLOSE ((I+8),STATUS='KEEP')
272 90         CONTINUE
273
274         RETURN
275         ENDIF
276

```

```

277      CLOSE (2)
278
279 31    FORMAT (5X,' NOTE that the new FILE NAME will have the',
280      + ' extension .DAT')
281 32    FORMAT (5X,' or n.DAT in cases where there are more than one/'
282      + ' channel read at a time, n represents the channel number'//)
283 33    FORMAT (5X,' NAME for the new FILE (4 char) -->\')
284 250   FORMAT (/15X,29(*))/
285      + 15X,' File 'A9,' is ready */15X,29(*))
286 300   FORMAT (4X,A11,1X,A25/)
287 400   FORMAT (' Channel no 'I1,' contains 'I4,' bins'/)
288 450   FORMAT (' Start of scan: 'I4,5X,' End of scan: 'I4/)
289 451   FORMAT (' Resolution: 'F7.3,1X,A4,'/bin'/)
290 500   FORMAT (8X,A4,11X,'volts'/)
291 600   FORMAT (6X,I4,4X,'=> F10.3)
292 605   FORMAT (15X,59(*))
293 610   FORMAT (15X,'*2X,EXCITATION WAVELENGTH: 'F6.2,' nm = ',
294      + F9.2,' cm-1',5X,'*')
295 615   FORMAT (10X,'X AXIS',9X,'Y AXIS')
296 620   FORMAT (5X,F10.2,5X,F10.3)
297 625   FORMAT (/15X,'WRITING ASCII FILE      BE PATIENT'/)
298 627   FORMAT (/15X,'BECAUSE NBINS = 'I5,'EVERY 'I2,' POINTS',
299      + ' IS USED IN THIS FILE'/)
300 630   FORMAT (15X,'*',15X,'DATA FILE NAME: 'A8,16X,'*')
301 631   FORMAT (15X,'*',15X,'DATA FILE NAME: 'A9,16X,'*')
302 640   FORMAT (15X,'*2X,START OF SCAN: 'F9.3,5X,'END OF SCAN: ',
303      + F9.3,3X,'*')
304 650   FORMAT (15X,'*2X,FIRST X VALUE: 'F8.3,6X,'LAST X VALUE: ',
305      + F8.3,4X,'*')
306 660   FORMAT (15X,'*2X,MIN Y VALUE: 'F8.3,6X,'MAX Y VALUE: ',
307      + F8.3,4X,'*')
308 700   FORMAT (/)
309 800   FORMAT (5X,' -999',5X,' -999')
310 900   FORMAT (5X,F10.3)
311 918   FORMAT (5X,F10.2,5X,F10.3)
312
313      RETURN
314      END

```

COPIE Local Symbols

Name	Class	Type	Size	Offset
NUM	param			0006
X	local	REAL*4	32000	0000
TEXT	local	CHAR*25	25	0002
X0	local	REAL*4	4	001c
I	local	INTEGER*4	4	0020
J	local	INTEGER*4	4	0024
START	local	REAL*4	4	0028
LDIF	local	INTEGER*4	4	002c
DNAME	local	REAL*4	4	0030

RES	local REAL*4	4	0034
DFINL	local REAL*4	4	0038
RAMANA	local CHAR*4	4	003c
VFFT	local REAL*4	4	0040
EXCW	local REAL*4	4	0044
WEXC	local REAL*4	4	0048
SCALIN	local REAL*4	4	004c
RDAT	local CHAR*5	15	7d00
RNAME	local CHAR*10	30	7d0f
Y	FILEB REAL*8	98400	0000
YY	FILEB REAL*8	32800	8060
MAX	FILEB REAL*8	8	0080
MIN	FILEB REAL*8	8	0088
DEP	FILEB REAL*4	4	0090
FIN	FILEB REAL*4	4	0094
NBINS	FILEB INTEGER*4	4	0098
NCHNS	FILEB INTEGER*4	4	009c
IDEP	FILEB INTEGER*4	4	00a0
IFIN	FILEB INTEGER*4	4	00a4
FACTOR	FILEB INTEGER*4	4	00a8
XBIN	FILEB INTEGER*4	4	00ac
NBIN	FILEB INTEGER*4	4	00b0
OLDPT	FILEB INTEGER*2	24600	00b4
ACT	FILEB	3	60cc
ANS	FILEB	3	60cf
UNITS	FILEB	4	60d2
UNITY	FILEB	4	60d6
RAWDAT	FILEB LOGICAL*4	4	60da
YESCL	FILEB LOGICAL*4	4	60de
RATIO	FILEB LOGICAL*4	4	60e2
ESC	FILEB LOGICAL*4	4	60e6
SCALE	FILEB REAL*4	4	60ea
SINT	FILEB REAL*4	4	60ec

Global Symbols

Name	Class	Type	Size	Offset
CLIP	extern	***	***	***
COPIE	FSUBRT	***	***	0000
FILEB	common		155890	0000
NORMAL	extern	***	***	***

C.2.4 BGETD.FOR

```

1      SUBROUTINE GETD(DNAME)
2
3      INCLUDE 'FILEB.TXT'
4
5      CHARACTER    DATAN*9,BLANK*10,DNAME*12

```

```

6      LOGICAL      EXIST
7      DATA BLANK/      /
8
9 C    READING OF DATA FILE NAME WITHOUT EXTENSION
10 1    WRITE (*,100)
11      READ (*,(A9)) DATAN
12
13      DO 10 I=1,9
14      IF ((DATAN(I:I).EQ.' ').OR.(DATAN(I:I).EQ.' ')) THEN
15      N = I - 1
16      DNAME=DATAN
17      DNAME(I:I+4)='.DAT'
18      DNAME(I+5:12)=BLANK(I:10-I)
19      GOTO 99
20      ENDIF
21 10    CONTINUE
22
23 99    INQUIRE (FILE=DNAME,EXIST=EXIST)
24      IF (.NOT.EXIST) THEN
25      WRITE (*,300) DNAME
26      READ (*,(A)) ANS
27      IF (ANS.EQ.'X'.OR.ANS.EQ.'x') THEN
28      STOP
29      ELSE
30      GOTO 1
31      ENDIF
32      ENDIF
33      WRITE (*,200) DNAME,N
34
35 100    FORMAT (/10X,'IN 7 CHARACTERS OR LESS,/'
36      + 10X,'WHAT IS THE DATA FILE NAME? -> \')
37 200    FORMAT (/10X,A12,' IS A 'J1,' LETTER FILE')
38 300    FORMAT (10X,A12,' COULD NOT BE FOUND')
39      + 1X,'DO YOU WANT TO TRY AGAIN OR EXIT TO GET THE FILE IN/'
40      + 1X,'      SUBDIRECTORY? T/X -> \')
41
42 1000  RETURN
43      END

```

GETD Local Symbols

Name	Class	Type	Size	Offset
DNAME	param			0006
I	local	INTEGER*4	4	0002
EXIST	local	LOGICAL*4	4	0006
BLANK	local	CHAR*10	10	0008
N	local	INTEGER*4	4	000a
DATAN	local	CHAR*9	9	000e
Y	FILEB	REAL*8	98400	0000
YY	FILEB	REAL*8	32800	8060

MAX	FILEB	REAL*8	8	0080
MIN	FILEB	REAL*8	8	0088
DEP	FILEB	REAL*4	4	0090
FIN	FILEB	REAL*4	4	0094
NBINS	FILEB	INTEGER*4	4	0098
NCHNS	FILEB	INTEGER*4	4	009c
IDEP	FILEB	INTEGER*4	4	00a0
IFIN	FILEB	INTEGER*4	4	00a4
FACTOR	FILEB	INTEGER*4	4	00a8
XBIN	FILEB	INTEGER*4	4	00ac
NBIN	FILEB	INTEGER*4	4	00b0
OLDPT	FILEB	INTEGER*2	24600	00b4
ACT	FILEB		3	60cc
ANS	FILEB		3	60cf
UNITS	FILEB		4	60d2
UNITY	FILEB		4	60d6
RAWDAT	FILEB	LOGICAL*4	4	60da
YESCL	FILEB	LOGICAL*4	4	60de
RATIO	FILEB	LOGICAL*4	4	60e2
ESC	FILEB	LOGICAL*4	4	60e6
SCALE	FILEB	REAL*4	4	60ea
SINT	FILEB	REAL*4	4	60ee

Global Symbols

Name	Class	Type	Size	Offset
FILEB	common		155890	0000
GETD	FSUBRT	***	***	0000

C.2.5 BINUMFOR

```

2      SUBROUTINE BINUM
3
4      $INCLUDE:'FILEB.TXT'
5
6      WRITE (*,100) UNITS
7      READ (*,*) VALUE
8      NBINUM = INT((VALUE - SINT)/SCALE)
9      WRITE (*,200) VALUE,UNITS,NBINUM
10
11 100  FORMAT (/10X,'WHICH',A4,' TO BE CHANGED IN BIN NUMBER? -->')
12 200  FORMAT (/10X,'VALUE ',F7.3,1X,A4,' ==> BIN NUMBER ',I4/)
13
14      RETURN
15      END

```

BINUM Local Symbols

Name	Class	Type	Size	Offset
NBINUM.....	local	INTEGER*4	4	0002
VALUE.....	local	REAL*4	4	0006
Y.....	FILEB	REAL*8	98400	0000
YY.....	FILEB	REAL*8	32800	8060
MAX.....	FILEB	REAL*8	8	0080
MIN.....	FILEB	REAL*8	8	0088
DEP.....	FILEB	REAL*4	4	0090
FIN.....	FILEB	REAL*4	4	0094
NBINS.....	FILEB	INTEGER*4	4	0098
NCHNS.....	FILEB	INTEGER*4	4	009c
IDEP.....	FILEB	INTEGER*4	4	00a0
IFIN.....	FILEB	INTEGER*4	4	00a4
FACTOR.....	FILEB	INTEGER*4	4	00a8
XBIN.....	FILEB	INTEGER*4	4	00ac
NBIN.....	FILEB	INTEGER*4	4	00b0
OLDPT.....	FILEB	INTEGER*2	24600	00b4
ACT.....	FILEB		3	60cc
ANS.....	FILEB		3	60cf

BINUM Local Symbols

Name	Class	Type	Size	Offset
UNITS.....	FILEB		4	60d2
UNITY.....	FILEB		4	60d6
RAWDAT.....	FILEB	LOGICAL*4	4	60da
YESCL.....	FILEB	LOGICAL*4	4	60de
RATIO.....	FILEB	LOGICAL*4	4	60e2
ESC.....	FILEB	LOGICAL*4	4	60e6
SCALE.....	FILEB	REAL*4	4	60ea
SINIT.....	FILEB	REAL*4	4	60ee

Global Symbols

Name	Class	Type	Size	Offset
BINUM.....	FSUBRT	***	***	0000
FILEB.....	common		155890	0000

C.2.6 BNAME.FOR

```

1      SUBROUTINE BNAME(DATAN)
2
3      CHARACTER DATAN*9,BLANK*10,DNAME*12
4      COMMON      DNAME
5
6      DATA BLANK/
7

```

```

8      DO 10 I=1,9
9      IF ((DATAN(I:I).EQ.' ').OR.(DATAN(I:I).EQ.' ')) THEN
10     N = I - 1
11     DNAME=DATAN
12     DNAME(I:I+4)='.DAT'
13     DNAME(I+5:12)=BLANK(I:10-I)
14     GOTO 99
15     ENDIF
16 10   CONTINUE
17
18 99   WRITE (*,100) DNAME,N
19
20 100  FORMAT (1X,A12,' IS A 'I1,' LETTER FILE')
21
22     END

```

BNAME Local Symbols

Name	Class	Type	Size	Offset
DATAN	param			0006
I	local	INTEGER*4	4	0002
BLANK	local	CHAR*10	10	0006
N	local	INTEGER*4	4	0006
DNAME	COMMQQ		12	0000

Global Symbols

Name	Class	Type	Size	Offset
BNAME	FSUBRT	***	***	0000
COMMQQ.	common		12	0000

C.2.7 BNORMALFOR

```

2 C    NORMALIZATION OR ORIGINAL DATA SUBROUTINE
3      SUBROUTINE NORMAL(NORM,I)
4  *
5  $INCLUDE:FILEB.TXT
6
7 C    FIND MAXIMUM AND MINIMUM VALUE IN FILE
8      MAX = Y(I,1)
9      MIN = Y(I,1)
10     DO 60 J=IDEP,IFIN,XBIN
11     IF (Y(I,J).GT.MAX) THEN
12     MAX = Y(I,J)
13     N = J
14     XN = REAL(N + IDEP)*SCALE + SINT
15     ENDIF

```

```

16      IF (Y(I,J).LT.MIN) THEN
17      MIN = Y(I,J)
18      M = J
19      XM = REAL(M + IDEP)*SCALE + SINI
20      ENDIF
21 60    CONTINUE
22
23      MAX = MAX - MIN
24
25      IF (NORM.EQ.3) GOTO 1000
26
27  C    NORMALIZE BUT FIRST PUT DATA IN ANOTHER FILE Yy
28      IF (NORM.EQ.1) THEN
29      DO 50 J=1,NBINS
30 50    Yy(I,J) = Y(I,J)
31      DO 70 J=IDEP,IFIN,XBIN
32      Y(I,J) = (Y(I,J) - MIN)/MAX
33 70    CONTINUE
34  C    MAKE SURE FILE IS MADE OF DIFFERENT VALUES
35      IF (MAX.NE.0.0) GOTO 65
36      WRITE (*,100)
37      RETURN
38 65    CONTINUE
39      WRITE (*,200) XN,UNITY,N,XM,UNITY,M
40      RETURN
41      ENDIF
42
43  C    PUT BACK DATA IN WORKING FILE
44      IF (NORM.EQ.2) THEN
45      DO 80 J=1,NBINS
46      Y(I,J) = Yy(I,J)
47 80    CONTINUE
48      WRITE (*,300)
49      ENDIF
50
51 100   FORMAT (/10X, ' DIVISION BY 0  — CHECK FILE  —'//)
52 200   FORMAT (/10X, ' NORMALIZED DATA'//10X,'Maximum (1) is at ',
53      + F10.3,1X,A4,' or bin number no ',I4/10X,' and'/
54      + 10X,'Minimum (0) is at ',F10.3,1X,A4,' or bin number no ',I4)
55 300   FORMAT (/10X, ' ORIGINAL DATA'//)
56
57 1000  RETURN
58      END

```

NORMAL Local Symbols

Name	Class	Type	Size	Offset
I.....	param		0006	
NORM.....	param		000a	
J.....	local	INTEGER*4	4	0002

M	local	INTEGER*4	4	0006
N	local	INTEGER*4	4	000a
XM	local	REAL*4	4	000e
XN	local	REAL*4	4	0012
Y	FILEB	REAL*8	98400	0000
YY	FILEB	REAL*8	32800	8060
MAX	FILEB	REAL*8	8	0080
MIN	FILEB	REAL*8	8	0088
DEP	FILEB	REAL*4	4	0090
FIN	FILEB	REAL*4	4	0094
NBINS	FILEB	INTEGER*4	4	0098
NCHNS	FILEB	INTEGER*4	4	009c
IDEP	FILEB	INTEGER*4	4	00a0
IFIN	FILEB	INTEGER*4	4	00a4
FACTOR	FILEB	INTEGER*4	4	00a8
XBIN	FILEB	INTEGER*4	4	00ac
NBIN	FILEB	INTEGER*4	4	00b0
OLDPT	FILEB	INTEGER*2	24600	00b4
ACT	FILEB		3	60cc
ANS	FILEB		3	60cf
UNITS	FILEB		4	60d2
UNITY	FILEB		4	60d6
RAWDAT	FILEB	LOGICAL*4	4	60da
YESCL	FILEB	LOGICAL*4	4	60de
RATIO	FILEB	LOGICAL*4	4	60e2
ESC	FILEB	LOGICAL*4	4	60e6
SCALE	FILEB	REAL*4	4	60ea
SINT	FILEB	REAL*4	4	60ec

Global Symbols

Name	Class	Type	Size	Offset
FILEB	common		155890	0000
NORMAL	FSUBRT	***	***	0000

C.3 SPEWBIN.FOR

```

1  PROGRAM BINSWAP
2  C  PROGRAM TO CHANGE A FORMATTED ASCII FILE FROM THE FFT
3  C  ALLIGATOR PROGRAM TO A BINARY FILE THAT CAN BE SEEN USING
4  C  GRAPHICS PACKAGE OF SR245 INTERFACE MODULE
5
6  REAL      Y(4100)
7  INTEGER*4  FACTOR,NBIN
8  INTEGER*2  OLDPT(4100)
9  LOGICAL*4  EXIST,YESCL
10 CHARACTER  UNITS*4,FNAME*8,RNAME*8
11
12  DATA  SCALING/1.0,START/1.0/

```

```

13      DATA  UNITS/cm-1/,RNAME/BINF.DAT/,EXIST/.TRUE./
14      DATA  YESCL/.FALSE./
15
16      WRITE (*,100)
17      READ (*,'(A8)')FNAME
18      WRITE (*,110)
19      READ (*,*) NP
20
21      OPEN (1,FILE=FNAME,STATUS='OLD')
22      OPEN (2,FILE=RNAME,STATUS='NEW',FORM='BINARY',IOSTAT=NCHK)
23 C     READ ASCII FILE FROM ALLIGATOR PROGRAM
24      READ (1,200) (Y(J),J=1,NP)
25          DO 3 J=1,NP
26              OLDPT(J)=INT(Y(J)*400.)
27 3      CONTINUE
28 C     WRITE BINARY FILE
29          write (*,*) '
30          write (*,*) '   Writing a binary file '
31          write (*,*) '
32      FACTOR = 1
33 C     2105 (LN of non-positive argument) IN PROGRAM
34 C     \SR245\SR265.EXE
35      NBIN = DBLE(NP)
36      WRITE (2) EXIST,FACTOR,NBIN
37      DO 39 J=1,NP
38      WRITE (2) OLDPT(J)
39 39    CONTINUE
40      WRITE (2) YESCL,SCALING,START,UNITS
41
42 100    FORMAT(1X,'INPUT ASCII FILE NAME (8 char.) -> \')
43 110    FORMAT(1X,'INPUT NUMBER OF POINTS IN FILE -> \')
44 200    FORMAT (E12.6)
45
46      CLOSE (1,STATUS='KEEP')
47      CLOSE (2,STATUS='KEEP')
48
49      END

```

main Local Symbols

Name	Class	Type	Size	Offset
OLDPT	local	INTEGER*2	8200	000a
SCALIN	local	REAL*4	4	0022
START	local	REAL*4	4	0026
UNITS	local	CHAR*4	4	002a
RNAME	local	CHAR*8	8	002c
EXIST	local	LOGICAL*4	4	0036
YESCL	local	LOGICAL*4	4	003a
J	local	INTEGER*4	4	2012
Y	local	REAL*4	16400	2016

NP.....	local	INTEGER*4	4	6026
NCHK.....	local	INTEGER*4	4	602a
FNAME.....	local	CHAR*8	8	602c
NBIN.....	local	INTEGER*4	4	6036
FACTOR.....	local	INTEGER*4	4	603a

Global Symbols

Name	Class	Type	Size	Offset
main.....	FSUBRT	***	***	0000

C.4 SID.FOR

```

1 C      DEC. 9, 1986
2 C      PARAMETERS USED TO IDENTIFY THE SCAN.
3 C
4 C
5 C      EXPP(1) = START OF SCAN      -> X0
6 C      EXPP(2) = END OF SCAN        -> DFINL
7 C      EXPP(3) = RESOLUTION IN UNITS/bin -> EXPP(3)
8 C      EXPP(4) = NUMBER OF POINT IN SCAN -> KOFBINS MAX:8000
9 C      GAIN = BOXCAR SENSITIVITY
10 C     EXPP(6) = LASER POWER IN mW
11 C     EXPP(7) = LASER WAVELENGTH IN nm > EVENTUALLY THIS
12 C     EXPP(8) = BOXCAR DELAY IN UNIT | WILL GO INTO THE
13 C     EXPP(9) = NUMBER OF AVERAGED SAMPLES
14 C     EXPP(10)= INPUT SLIT IN um    _1 FINAL PLOT
15
16
17
18 CHARACTER  ANS*1,UNIT*4,GAIN*5,COMMENTS*35~
19 COMMON    EXPP(10),X0,DFINL,UNIT,GAIN,COMMENTS
20
21
22 1000 WRITE (*,*) 'START OF SCAN'
23     READ (*,*) X0
24
25 1001 WRITE (*,*) 'END OF SCAN'
26     READ (*,*) DFINL
27
28 C      CHECK FOR MONOCROMATOR LIMITS
29 IF (X0.LT.12700.0.OR.X0.GT.25900.0) THEN
30     WRITE (*,10)
31     WRITE (*,12)
32     GOTO 1000
33     ENDIF
34 IF (DFINL.LT.12700.0.OR.DFINL.GT.25900.0) THEN
35     WRITE (*,10)
36     WRITE (*,12)

```

```

37         GOTO 1001
38     ENDIF
39
40     WRITE (*,*) 'RESOLUTION / STEP SIZE'
41     READ (*,*) EXPP(3)
42
43     WRITE (*,*) 'LASER POWER IN mW'
44     READ (*,*) EXPP(6)
45
46     WRITE (*,*) 'LASER WAVELENGTH IN nm'
47     READ (*,*) EXPP(7)
48
49     WRITE (*,*) 'UNITS FOR BOXCAR DELAY '
50     READ (*,*(A4)) UNIT
51     WRITE (*,31) UNIT
52     READ (*,*) EXPP(8)
53
54     WRITE (*,*) 'BOXCAR SENSITIVITY IN mV   e.g. 50/20'
55     READ (*,*(A5)) GAIN
56
57     WRITE (*,*) 'AVERAGING SAMPLES (30 or 100) '
58     READ (*,*) EXPP(9)
59
60     WRITE (*,*) 'SIZE OF INPUT SLITS IN MICROMETERS'
61     READ (*,*) EXPP(10)
62
63     WRITE (*,*) 'OTHER COMMENTS? Y/N'
64     READ (*,*(A1)) ANS
65     IF (ANS.EQ.'Y'.OR.ANS.EQ.'y') THEN
66         WRITE (*,*) 'WRITE COMMENTS IN 35 char. MAX'
67         WRITE (*,*) 'i.e. solvent, concentration, and so on'
68         READ (*,*(A35)) COMMENTS
69     ENDIF
70
71     EXPP(4) = ABS(DFINL - X0)/EXPP(3)
72
73     WRITE (*,*) 'DO YOU WANT TO MAKE ANY CHANGES? (Y/N) '
74     READ (*,*(A1)) ANS
75     IF (ANS.EQ.'Y'.OR.ANS.EQ.'y') GOTO 1000
76     WRITE (*,*) '
77     WRITE (*,*) 'DO YOU WANT TO WRITE INFORMATION'
78     WRITE (*,*) 'ON FILE FOR PLOTS ? '
79     WRITE (*,*) '
80     READ (*,*(A1)) ANS
81
82     IF (ANS.EQ.'Y'.OR.ANS.EQ.'y') THEN
83         CALL PARAMID (4)
84         WRITE (*,*) '
85     WRITE (*,*) 'THE INFORMATION FILE -> EXTENSION .RID'
86         WRITE (*,*) '
87     WRITE (*,*) 'HERE IS WHAT YOU HAVE ON FILE '
88         WRITE (*,*) '

```



```

89      CALL PARAMID(3)
90      ENDIF
91
92      WRITE (*,*) '
93      WRITE (*,*) ' DO YOU WANT A HARD COPY OR A DISPLAY'
94      WRITE (*,*) ' OF THE SUMMARY OF ALL PARAMETERS? (H/D) '
95      WRITE (*,*) '
96      READ (*, '(A1)) ANS
97      IF (ANS.EQ.'H'.OR.ANS.EQ.'h') CALL PARAMID (2)
98      IF (ANS.EQ.'D'.OR.ANS.EQ.'d') CALL PARAMID (1)
99
100 6    FORMAT(/)
101 10    FORMAT(15X,'*****'/)
102 11    FORMAT(30X,' DIAL READING'/)
103 12    FORMAT(25X,'OUT OF MONOCHROMATOR LIMITS',//,35X,'TRY AGAIN//
104      + 15X,'*****'/)
105 31    FORMAT(' BOXCAR DELAY IN ',A4)
106
107      END

```

main Local Symbols

Name	Class	Type	Size	Offset
ANS	local	CHAR*1	1	0002
UNIT	COMMQQ		4	0030
GAIN	COMMQQ		5	0034
COMMEN	COMMQQ		35	0039
EXPP	COMMQQ	REAL*4	40	0000
X0	COMMQQ	REAL*4	4	0028
DFINL	COMMQQ	REAL*4	4	002c

```

108 C    SUBROUTINE TO DISPLAY EXPERIMENTAL PARAMETERS ON SCREEN
109 C    THE FIRST TABLE INTITLED "LIST OF RAMAN PARAMATERS"
110 C    CONTAINS VALUES GIVEN BY THE OPERATOR AND
111 C    CALCULATED VALUES SUCH AS NUMBER OF POINTS -EXPP(4)-
112 C    AND RESOLUTION -EXPP(3)- WHEN NECESSARY.
113 C    THE SECOND TABLE INTITLED "QUESTIONS ANSWERS"
114 C    PROVIDES THE ANSWERS TO THE DATA ACQUISITION AND CONTROL
115 C    PROGRAM (JY265.EXE).
116
117      SUBROUTINE PARAMID (NUM)
118
119      CHARACTER  RNAME*9,RID*5,PARANAME*4,COMMENTS*35
120      CHARACTER  UNIT*4,GAIN*5,XDAT*5,XNAME*9
121      COMMON     EXPP(10),X0,DFINL,UNIT,GAIN,COMMENTS
122
123      OPEN (2, FILE = 'PRN')
124
125
126      IF (NUM.EQ.1) THEN
127          WRITE (*,8) COMMENTS

```

```

128      WRITE (*,3)
129      WRITE (*,2)
130      WRITE (*,110) X0
131      WRITE (*,120) DFINL
132      WRITE (*,130) EXPP(3)
133      WRITE (*,140) EXPP(4)
134      WRITE (*,160) EXPP(6)
135      WRITE (*,170) EXPP(7)
136      WRITE (*,180) EXPP(8),UNIT
137      WRITE (*,190) GAIN
138      WRITE (*,192) EXPP(9)
139      WRITE (*,200) EXPP(10)
140
141      WRITE (*,1)
142      WRITE (*,4)
143      WRITE (*,2)
144      WRITE (*,41) EXPP(4),EXPP(3)
145      WRITE (*,2)
146      ENDIF
147
148      IF (NOM.EQ.2) THEN
149      WRITE (*,*) ' *****
150      WRITE (*,*) ' - MAKE SURE THAT THE * PRINTER * IS ON'
151      WRITE (*,*) ' *****
152      WRITE (*,*) '
153          WRITE (2,6)
154          WRITE (2,6)
155          WRITE (2,5)
156          WRITE (2,6)
157          WRITE (2,7)
158          WRITE (2,8) COMMENTS
159          WRITE (2,1)
160          WRITE (2,2)
161          WRITE (2,3)
162          WRITE (2,2)
163          WRITE (2,110) X0
164          WRITE (2,120) DFINL
165          WRITE (2,130) EXPP(3)
166          WRITE (2,140) EXPP(4)
167          WRITE (2,160) EXPP(6)
168          WRITE (2,170) EXPP(7)
169          WRITE (2,180) EXPP(8),UNIT
170          WRITE (2,190) GAIN
171          WRITE (2,192) EXPP(9)
172          WRITE (2,200) EXPP(10)
173
174          WRITE (2,2)
175          WRITE (2,1)
176          WRITE (2,2)
177          WRITE (2,4)
178          WRITE (2,2)
179          WRITE (2,41) EXPP(4),EXPP(3)

```

```

180      WRITE (2,2)
181      WRITE (2,1)
182      WRITE (2,6)
183      WRITE (2,6)
184      ENDIF
185
186      IF (NUM.EQ.3) THEN
187      WRITE (*,250) RNAME
188      WRITE (*,300) EXPP(6),EXPP(8),UNIT,EXPP(7),EXPP(9),EXPP(10)
189      WRITE (*,8) COMMENTS
190      ENDIF
191
192      IF (NUM.EQ.4) THEN
193      WRITE (*,100)
194      READ (*,(A4)) PARANAME
195      RID = 'RID0'
196      XNAME(1:4)=PARANAME
197      RNAME(1:4)=PARANAME
198      RNAME(5:4+5)=RID
199      OPEN (4,FILE=RNAME,STATUS='NEW')
200      WRITE (4,300) EXPP(6),EXPP(8),UNIT,EXPP(7),EXPP(9),EXPP(10)
201      WRITE (4,8) COMMENTS
202
203      ENDIF
204
205 1      FORMAT(15X,'*****')
206 2      FORMAT(15X,'*')
207 3      FORMAT(15X,'* LIST OF RAMAN SPECTRUM PARAMETERS *')
208 4      FORMAT(15X,'* QUESTIONS ANSWERS *')
209 5      FORMAT(15X,'DATE : _____')
210 6      FORMAT(/)
211 7      FORMAT(15X,'SAMPLE : _____')
212 +      ,24X,' _____')
213 8      FORMAT(15X,'COMMENTS: 'A35)
214 41     FORMAT(15X,'* Numbers of channels? 1 *')
215 +      15X,'* Numbers of bins? 'F7.0,'*')
216 +      15X,'* Time Scans? No *')
217 +      15X,'* Label X-axis? Yes (cm-1) *')
218 +      15X,'* How many cm-1/bin ? 'F9.1,4X,'*')
219 +      15X,'* bin 1 = ? 1 *')
220 +      15X,'* Other SR245 Commands? Yes *')
221 +      15X,'* At Each Point: T30 *')
222 100    FORMAT (' Give a NAME to the FILE (4 char) ->\')
223 110    FORMAT(15X,'* 5X,START OF SCAN = 'F9.1,1X,'cm-1',6X,
224 +      *)
225 120    FORMAT(15X,'* 5X,END OF SCAN = 'F9.1,1X,'cm-1',6X,
226 +      *)
227 130    FORMAT(15X,'* 5X,RESOLUTION = 'F5.1,1X,
228 +      'cm-1/point *')
229 140    FORMAT(15X,'* 5X,NUMBER OF POINTS = 'F7.0,13X,*)
230 160    FORMAT(15X,'* 5X,LASER POWER = 'F7.2,' mW',10X,*)
231 170    FORMAT(15X,'* 5X,LASER WAVELENGTH = 'F7.1,' nm',10X,*)

```

```

232 180    FORMAT(15X,'5X,BOXCAR DELAY    = 'F7.2,1X,A4,8X,')
233 190    FORMAT(15X,'5X,BOXCAR SENSITIVITY= 'A5,' mV',10X,')
234 192    FORMAT(15X,'5X,AVERAGED SAMPLES = 'F5.0,13X,')
235 200    FORMAT(15X,'5X,INPUT SLITS    = 'F7.0,' um',10X,')
236 250    FORMAT(15X,A8/)
237 300    FORMAT(' LASER POWER          : 'F7.2,' mW',4X,
238      +   'BOXCAR DELAY          : 'F7.2,1X,A4/
239      +   'EXCITATION WAVELENGTH : 'F7.2,' nm',
240      +   4X,'AVERAGED SAMPLES : 'F5.0,7X/
241      +   'INPUT SLITS SIZE      : 'F7.0,' um',37X/)
242 310    FORMAT (/5X,F6.2,' nm = 'F8.2,' cm-1'/)
243 333    FORMAT (1X,F10.2)
244
245        CLOSE (2)
246        CLOSE(4,STATUS='KEEP')
247        RETURN
248        END

```

PARAMI Local Symbols

Name	Class	Type	Size	Offset
NUM	param			0006
RID	local	CHAR*5	5	0004
XDAT	local	CHAR*5	5	000a
PARANA	local	CHAR*4	4	0010
RNAME	local	CHAR*9	9	0014
XNAME	local	CHAR*9	9	001e
COMMEN	COMMQQ		35	0039
UNIT	COMMQQ		4	0030
GAIN	COMMQQ		5	0034
EXPP	COMMQQ	REAL*4	40	0000
X0	COMMQQ	REAL*4	4	0028
DFINL	COMMQQ	REAL*4	4	002c

Global Symbols

Name	Class	Type	Size	Offset
COMMQQ	common		92	0000
PARAMI	FSUBRT	***	***	0401
main	FSUBRT	***	***	0000

END

29/06/89

FIN