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Automatic Identification and Enumeration of Algae.

Final Report.

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CONTENTS

CONTRACT DESCRIPTION

Prior knowledge of the likely behaviour of algae in a reservoir would allow remedial action to be taken at the most cost effective point in time. Information on the species composition is an important criterion in predicting this behaviour. Although automatic instrumentation is available to count and size particulate material, at the present time taxonomic separation is only possible using manual identification with an optical microscope. The objective of this contract has been to assess the potential of algal fluorescence as a basis for the identification of individual algal species in automatic equipment. The programme of work includes the building of an instrument for the acquisition of total fluorescence spectra and the comparison of spectra from several different algal species to determine the level to which it would be practically possible to differentiate between species automatically.

ABSTRACT

An instrument was built which was capable of recording total fluorescence spectra from live algal suspensions. Spectra were obtained from three distinctive species of algae from each of three different classes (green algae, diatoms, blue-green algae). Comparison with published absorption spectra showed that considerable fine structure was present but it was not apparent in any of the visual representations of the total data set. Simple comparisons highlighted spectral regions which allowed differentiation between classes but species differentiation will require automated multivariate techniques. Further work is proposed to find the minimum amount of information which is required to distinguish algae by fluorescence, before incorporating appropriate sensors on a flow cytometer algal counter - sizer - speciation instrument being developed at Strathclyde University.

INTRODUCTION

The ability to automatically identify and enumerate algae in a mixed culture or in a natural population would be a major advance in environmental research. Major financial savings could be made by Water Authorities in the cost of water treatment if 'real-time' information on algal species and numbers were available for use in predictive models of population change. Algal types present in mixed cultures are currently identified by traditional taxonomic techniques, which are extremely time consuming and costly.

Several automatic techniques are under development. The combination of flow cytometry and multi-parametric cell sorting, reviewed by Horan and Wheeless (1977), has found application through the work of Yentsch et al. (1983) and promises some degree of differentation of algae but, at present, is still dependent on confirmatory tests such as epifluorescence microscopy. Brahma et al. (1983) have developed a resonance Raman method for identification of algae at class level from culture whilst Krause et al. (1982) have differentiated between algal phyla and identified populations in freshwater using delayed fluorescence techniques.

Different algal species are able to exploit different environmental conditions.One method by which this is achieved is by utilising different spectral regions of the incoming light.Pigments are arranged in photosystems, constructed to absorb light from many wavelengths and channel it into the chlorophyll system. Accordingly different algae would be expected to contain different combinations of pigments. 'Waste light' of lower energy (higher wavelength) is often emitted as fluorescence at wavelengths indicative of the contributing pigments. The measurement of pigment concentrations by fluorescence is inherently very sensitive as

absolute intensities are used rather than the difference of two intense signals as is the case with absorbance.

The concept of total fluorescence measurement, whereby a matrix of excitation and emission wavelength 'co-ordinates' and an associated fluorescence intensity are used to produce topographical relief in the form of a contour map, was pioneered by Lloyd (1971) as a forensic technique in the identification of complex oils. Different fingerprints were produced depending on the origin of the oil. John and Soutar (1981) developed this technique further to apply to spills from oil tankers using synchronous scanning to produce better resolution.

In this work we aimed to extend total fluorescence methodology to serve in the identification of freshwater algae, which, by their very nature, contain a host of fluorescent pigments in varying relative proportions, depending on class or species. Initial studies in this laboratory involving manual recording and plotting of data were promising. Further, more detailed work which necessitated the automation of data collection, data reduction and data presentation is reported herein.

SPECTROMETER MODIFICATIONS

i) General description

The basic apparatus was designed and built in-house by C.R.Cunningham and M.J.Lee and occupied the first 12 months of the contract. A detailed electronic description is included in appendix 1.

For a small capital investment the existing Perkin Elmer model 204 fluorescence spectometer could be updated to carry out the task. Data storage and overall control could be effected by interfacing to an Apple IIe microcomputer. An interface was purpose built to control the data collection functions on the spectrometer. Very little alteration was required to the spectrometer itself, it being a precondition that the instrument could continue to be used in its previous manual mode if required. Figure 1 shows the final instrumental assembly.

ii) Minimum Wavelength Increment.

The instrument bandwidth was fixed by the manufacturers at 10nm. Any attempt to use wavelength increments less than this value would increase the number of data points with no enhancement of definition. At 10nm increments the data set for the range 220 - 780nm on both monochromators produced a 53 * 53 point matrix.

iii)Scan Speed tests.

The total time taken for a scan of a given algal sample had to be kept low, preferably below 30 minutes, but certainly below one hour, as physiological responses to the excitation light would probably induce a change in fluorescence characteristics over periods greater than this. Initial studies, using a standard polythene block impregnated with tetraphenyl butadiene, with the scan speed variable over the range 1-9 (2hr 15min - 15min for a

scan) showed that the system was too inflexible and that excessive peak smoothing was occuring at all speeds greater than 1 (Figure 2). Modifications allowed speed control over a range 1-99 and finer control of the wavelength interval. Reduction of the capacitors associated with the gain control to 1/10 th of the original value reduced the scan time to approximately 40 min for a 53 * 53 matrix of data with no obvious smoothing taking place (figure 3). Although the exchange led to an increase in the system noise, it was effectively damped by the a/d converter (Appendix 1, 2.7).

iv) Wavelength Accuracy.

Rapid data collection necessitated a fast monochromator rotation speed and demanded an equally fast photomultiplier response time. Any lag in the system would produce an apparent wavelength shift. Emission spectra of a standard polythene block impregnated with Rhodamine B were recorded at the minimum wavelength increment (3.7nm.) using scan speeds of 10 (slow) and 90 (fast). The results (figure 3) show that scan speed had little effect on the relative position or intensities of the peaks (578nm, 3600 intensity units at speed 10 and 575nm, 3540 units at speed 90). When compared to the manufacturers standard spectrum, differences were within the instrument´s specification, i.e. +/- 5nm.

v) Drive Calibration.

For a given angular rotation of the stepping motor drive units a slightly different wavelength increment was produced on the emission and excitation monochromators. Appropriate factors were included in the data - handling program to calculate the correct wavelengths for each monochromator.

vi) System Stability.

The system itself could be a source of error in terms of drift in sensitivity and peak position. Instrument performance was checked by recording a series of replicate total fluorescence spectra of a standard Rhodamine B block. Four replicate spectra were recorded at hourly intervals, scanning the excitation monochromator from 400 - 600 nm and the emission monochromator from 500-700 nm, with a wavelength increment corresponding to 10nm. Identical spectra were produced.

vii) System Noise.

Confident identification of any possible areas of low fluorescence intensity would only be possible if the background noise was constant. Replicate spectra were recorded of a quartz cell containing deionised water, with the instrument settings at maximum sensitivity. The main features in the spectra arose from the Raman effect of water, which yields lower energy emissions, and a contribution from Rayleigh scattering, which is of unchanged frequency. The remainder of the data matrix was a genuine background which varied between 0 and 30 intensity units, depending on lamp conditions and photomultplier drift. This level of background variation was considered acceptable as it represented less than 1% of the full scale intensity.

viii) Quantum Correction of Spectra.

No correction was made to any spectrum for variation with wavelength of either lamp output (associated with the excitation spectra) or photomultiplier sensitivity and monochromator transmission factors (associated with emission spectra). This was not considered to be necessary for the purposes of this study. Relative spectra rather than absolute spectra are presented.

SAMPLE HOMOGENEITY

i) General.

The ability to maintain a homogeneous sample, i.e. evenly suspended algae, during the recording of a spectrum is vital to the production of reproducible sprectra for comparison between algal species / classes. Settling out, etc., which occurred during data collection cannot be modelled and corrected for mathematically. Sample homogeneity was maintained by continuously mixing the sample.

ii) Mixer selection.

Two systems were investigated:

a) An external reservoir was stirred continuously and a small subsample was pumped into the flow cell via a peristaltic pump (figure 4). Replicate spectra were recorded using the green alga Chlorella. This system had the advantage of removing the algal cells to a standard condition throughout the experiment thereby reducing the likelyhood of physiological changes taking place within the cell in response to the continuous light environment of the flow cell. However a reduction in spectral intensities with time showed that cells were settling at the bottom of the flow cell, leading to a dilution of the suspension.

b) A small, battery driven stirrer in the analysis cell (figure 5) induced quite high mixing rates. Comparison of distilled water spectra taken with or without the stirrer in position showed that a high level of scatter was being introduced by the stirrer. The scatter was reduced to an acceptable level by blackening the drive shaft of the stirrer. Spectra recorded after

different periods of time from the introduction of the sample into the cell showed a slight settling effect, but the rate was insignificant compared with the time required to record a spectrum.

SPECTRAL PRESENTATION

i) General.

Prior to the work reported here, total fluorescence spectra had only been recorded on solutions. ´In vivo´ measurement of algal fluorescence requires particulate material to be present in the analysis cell, increasing, by several orders of magnitude, the amount of scatter recorded in the spectrum. An example is given in figure 6, where a spectrum of deionised water and a non - fluorescent particle suspension are shown. The peak marked ´R´ is due to Raman absorption from the solvent. The major feature in the water spectrum is the Rayleigh scattering peak running from corner to corner, i.e. excitation wavelength = emission wavelength. When particulate material is present this scatter is increased to such an extent that subordinate peaks occur at emission wavelengths = excitation wavelengths *2 or /2. This is due to the production within the monochromator of small quantities of energy with wavelengths either a fraction (1/2, 1/4, etc.) or a multiple (2*, 3*, etc.) of the nominal wavelength. The intensity of the scattered light was so large that the instrument gain settings were determined by this feature in order to protect the photomultiplier tube, reducing the ability to record low intensity fluorescence peaks. A second effect of the scatter harmonics in particular is to obscure much of the region covered by the fluorescence spectrum, particularly when the simpler ´pseudo - 3D´ plots were used (see later). Before further progress could be made with real samples a means had to be found to reduce, or preferably eliminate, this scattering so that full instrumental gain settings could be employed to detect the lower intensity fluorescence signals. Several methods were

tried.

ii) Correction for scatter peaks.

a) External subtraction of non - fluorescent particle spectra.

Aqueous suspensions of non - fluorescent particles over the size range 3.4um to 85um were prepared from standard materials (Puff balls,3.4um; Pollen,11.0um; Ragweed,20.0um; Lycopodium, 24.6um; Corn,85.0um). A total spectrum was recorded for each sample. 11um pollen grains were selected for use in the remainder of the study because of their proximity to the size of many of the non - colonial algal cells under study. They were also found to remain in suspension much longer than the larger particles.

Spectra were recorded for five suspensions of the 11um particles, starting with an arbitrary concentration, c, and diluting a sub-sample 1:1 to produce a series ; c, c/2, c/4, c/8, c/16. Subtraction of a scatter only spectrum from 'real' spectra was very successful in some cases, but not in others. This was thought to be due to the difference in particle shapes between the algae and the correction particles. The lack of reproducibility between corrections made discernment between peaks unreliable.

b) Subtraction of the spectrum of bleached algae.

The natural extension of the previous work was to try and match the size and shape of the sample and correction suspension. 10ul of 10-14% sodium hypochlorite solution was added to the algal suspension in the analysis cell immediately after data collection. The sample was left for 10 minutes, with stirring, before recording the correction spectrum. Microscopic examination of the treated sample showed that the cells were intact but contained no pigmentation. Corrected spectra showed the same

variability as spectra corrected using non - fluorescent particles.

c) ´Fold - over´.

Figure 7 outlines the typical features of a square data set. The distribution of scattered light appears to be symmetrical about the diagonal, and fluorescent peaks only occur on one side of the diagonal. Subtraction of the intensity at a pair of coordinates on one side of the diagonal from the intensity of the mirror image in the diagonal (e.g. A-A1) should result in a spectrum containing only the fluorescence information. The triangular matrix produced by this method is shown in figure 8. Unfortunately the fine detail in the scatter peaks was found to be asymmetrical giving the same effect as for the other two methods.

d) Time resolution of spectra.

Given appropriate instrumentation it is possible to delay the measurement of fluorescent output, which is on a time scale of 10(-9) seconds, so that scattered light which lasts for only about 10(-15) seconds after removal of the excitation energy, does not register in the detector. With the constraints of time and money this was not possible.

e) Practical solution.

By this stage of the development we had gained sufficient experience to realise that the secondary scatter peaks were not covering any fluorescence information when used in conjunction with contour presentation (see later). As all fluorescence occurs at emission wavelengths > excitation wavelengths, the most intense scatter could be removed simply by collecting data from the higher emission wavelength half of the matrix. The emission

monochromator was moved to the excitation wavelength + 20nm at the start of each emission scan. In this mode the intense scattered light could never enter the photomultiplier tube, allowing the use of high gain values with no danger of damaging the instrument.

iii) Data plotting.

The data was plotted in several different ways with the aid of computer programs. Listings are given in Appendix 2.

a) Pseudo 3D plots

If we consider the matrix of intensity data to be a series of emission scans, one after another, with gradually increasing excitation wavelength, then we can plot the data as a series of line scans. If each line is offset up the page by a distance proportional to the excitation wavelength increment a pseudo 3D plot is produced (figure 9). Although this type of presentation is simple to computerise, the spectra are difficult to interpret quantitatively. High intensity secondary scatter swamps the fluorescence peaks which appear behind it.

b) Reverse pseudo 3D plots.

As the fluorescence peaks were behind the secondary scatter peaks in (a), the data were replotted using the same line spectrum approach but as if it was being viewed from behind (figure 10). This made viewing of the central region much easier but observation of fine structure within the spectra was almost impossible.

c) Excitation spectra.

Published excitation spectra of algae, e.g. Heaney, 1978, contained a great deal of detail which was not apparent on the pseudo 3D plots. In order to check that this detail was present

in our data sets, appropriate data were extracted manually and plotted using an ´in - house´ plotting routine. These plots correlated well with the published spectra (figure 11), confirming our suscpisions that pseudo 3D and reverse pseudo 3D plots were hiding valuable information.

d) Contour plots.

Contouring programs are quite complex and not applicable to micro computers. However, when other presentations were found to be inadequate, a simplified style of contour program was written (appendix 2). Instead of automatically plotting the boundaries, each data point is assigned a letter , depending on the range of values it falls within. The ranges can be altered to give linear, logarithmic or variable scales, and the number of contours can be varied. A different coloured pen is chosen for each range. The contours are drawn manually at a later stage. This is a rapid process and removes the difficulty of making the computer decide where the boundary runs. Considerable detail is easily visible and all further work used this style of plot (figure 12).

ALGAL STUDIES

i) Species selection

Nine common algae (Table 1), which were already available in culture, were chosen to test the hypothesis that algal species can be identified by their fluorescence properties. Species were selected from each of the three main groups of freshwater algae, i.e. Chlorophytes, Cyanophytes and Bacillaiophytes commonly called: Green Algae; Blue-green Algae (or cyanobacteria) and diatoms respectively. Within each class a further selection was made to cover a range of sizes and shapes, colonial and unicellular. The differences between the species on a taxonomic level were as great as could be encountered in natural populations and maximised the possibility of differentiating between a) class and b) species.

ii) Sample preparation.

The algae were supplied from the F.B.A. Culture Collection by G.H.M.Jaworski. Samples were grown as monocultures under constant light conditions. They were maintained in the log-growth phase until immediately prior to subsampling. Replicate samples were obtained by subsampling the same culture vessel with gentle agitation between extractions.

TOTAL FLUORESCENCE SPECTRA

i)Green Algae.

Due to the regular nature of the cells and colonies, clean spectra were obtained (figure 12a,b,c). Intense emission was observed in the 680nm region from excitation over the range 360-500nm and 630-680nm for both Chlorella and Dictyosphaerium. Pediastum has a more compact spectrum, exciting over the ranges 420-480nm and 660nm. Pediastrum also showed a double peak in the 420-480nm region, which was also evident in both chlorella and dictyosphaerium at lower sensitivities. The areas of absorption include all the major pigments which are expected in green algae.

ii) Blue-green algae.

In the contour plots (figure 13a,b,c) there is intense emission around 650-670nm for excitation at 630-640nm. A low intensity chlorophyll emission is also visible at 670-680nm from excitation at 360-440nm. These effects are more difficult to see in the spectrum of Aphanizomenon which is very noisey, probably a result of the colony shape. Comparison with the spectra for the green algae suggests the presence of low emission in the 560-620nm region for a range of excitation wavelengths, indicative of the phycobilins.

iii) Diatoms.

Stephanodiscus produces a ´clean´ spectrum, whereas the spectra of both Asterionella and Fragilaria contain a lot of noise which is probably due to the irregular colonial shapes of the latter two species. All three spectra (figure 14a,b,c) show intense chlorophyll emission at 680nm due to excitation from 380-480nm. This intensity remains fairly high up to an excitation wavelength of 550nm, a feature which is not shown by the green algae. There is

some evidence for a chlorophyll excitation at 650-670nm.

iv) Raman Peaks.

The Raman peak appears on all spectra above(figure 12, 13, 14) and in the stirred media only (figure 15). As it is caused by absorption of vibrational energy by the solvent (water), it might be expected to remain constant. This is not the case, however. Its intensity varies from spectum to spectrum. It is probably the effect of the scattered light, which increases the apparent volume of water which is being irradiated and, hence re-emitting the energy. More light is transmitted to the detector by this means, compared to solution spectra, and the intensity is dependent on the scattering properties of the suspension, i.e. particle size, concentraion and shape.

CONCLUSIONS AND FURTHER WORK.

i) After taking account of the many interactive effects of sample introduction, data collection and spectral presentation, which have been discussed earlier, the fluorimeter design was optimised to allow total fluorescence spectra to be obtained from algal suspensions. In the final configuration a minimum wavelength increment of 10nm was defined by the bandwidth of the commercial spectrometer used. In order to keep intense light out of the photomultiplier and allow high gain values to be used to pick out fluorescence peaks, primary scatter peaks were avoided by collecting data from a small triangular region of the full emission - excitation matrix, such that emission wavelength >= excitation wavelength + 20nm. Secondary scatter peaks were found not to be a problem if data matrices were presented as contour diagrams.

ii) Visual comparison of the nine spectra show that there are consistant differences between spectra of algae from the different classes, as might be expected from the different pigments which make up their photosystems (table 2). The most sensitive regions are indicated in figure 16. Yentsch and Phinney (1985) have suggested that ratios of peak intensities, at ex = 530nm; em = 685nm and ex = 450nm; em = 685nm, may be used to study class changes within a population. However the use of a single ratio results in a range of ratios from 0.1 - 0.9. Individual classes fit into narrow ranges approximately 0.1 wide. In a mixed population there is a strong possibility that a population dominated by a mixture of classes with ratios 0.8 -0.9 and 0.1 -0.2 would have a bulk parameter in the mid range, implying domination by a class which is only present in small quantities. The data presented in our work suggest that more than one ratio could be used to increase the selectivity of the method, possibly even giving an estimate of

the relative proportions of the different classes within a population. Further work is required to persue this approach.

iii) The intensity of the Raman peak is a function of algal numbers and it may be possible to use the peak intensity as a normalising factor to obtain crude estimates of spectral yield. The latter varies from species to species and may provide further information for identification.

iv) It is obvious that algal classes can be identified using fluorescence properties, but visual inspection is not sufficiently discriminating to prove conclusively that species can be identified with the same data. Recently Oldham et al. (1985) have published a paper which follows the exact path of much of our original programme. They used a Fourier - transform - based pattern recognition system to show that algal signatures are recognisable from total flourescence spectra, verifying our original hypothesis. However, their work was aimed at identifying the algal species within a mixed population, and has shown the discrimination to be very limited , such that it can only identify the dominant algal species in a two component mixture.

Our work was intended to prepare the way for automatic identification of individual algal cells within a flow-cytometer type sizing - counting - identification instrument. Dr. Alex Cunningham of the University of Strathclyde has built such an instrument and his group is using diffraction patterns, i.e. shape, to speciate algae. They are very interested in using the extra power, which identification by flourescence should provide. In the one year extension of our work it is planned to look at the data we have in more detail to determine the minimum number of wavelength pairs which are required to speciate beyond class

level. With this information it will be possible to define the hardware required to instrument the fluorescence option. Assuming that the technology is available, we hope to work in conjunction with the Strathclyde group to modify their instrument accordingly.

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Table 1. Characteristic properties of selected algae.

Class	Species	FBA clone number	Shape		Mucilage	Diameter(μm)		Length (μm)	No per Colony	individual cell vol (μm^3)
			Unicellular	Colonial		single	colony			
Chlorophyta	Chlorella	L289	spherical	-	X	2-5	-	-	-	33(4)
Chlorophyta	Dictyosphaerium pulchellum	L246	spherical	ovoid in tetrads	✓	8-14	50-70	-	60-100	400
Chlorophyta	Pediastrum	L387	irregular-denticulate	disc-like	X	20-25	~100	-	16-32	16000
Cyanophyta	Anabaena flos aquae	L102	near spherical	coiled chains	X	6	80/100	-	40-200	60-100
Cyanophyta	Aphanizomenon	L373	filamentous	raft of filaments	X	2-5	60-300	60-80	<100	12
Cyanophyta	Microcystis aeruginosa	L305	spherical	clustered in sphere	✓	5	100-500	-	>100	60-100
Chrysophyta (Bacilliarophyta)	Asterionella formosa	L313	pennate	stellate	X	3	-	65	5-8	650
Chrysophyta (Bacilliarophyta)	Fragilaria crotonensis (flared)	L378	pennate	comb-like (double)	X	3-5	-	80	20-200	700
Chrysophyta (Bacilliarophyta)	Stephanodiscus hantszchii	L382	cylindrical (squat)	-	X	<10	-	10-11	-	<100

Table 2. Pigment types present in different algal classes.

Phylum (Class)	Pigments	Excitation wavelengths/nm	Emission wavelengths/nm
Chlorophyta	+ chlorophyll a	430-450[2], 663[1]	660-680[3]
	+ chlorophyll b	456[2], 634[1]	650[3]
	carotenoids	454[1]	
	xanthophylls (lutein neoxanthin zeazanthin)	448,432[1]	
Cyanophyta	chlorophyll a	430-450[1]	
	* phycobilins - phycoerythrins	490[2,3]	560-590[3]
	phycocyanin	610[2]	
Chrysophyta (Bacillariophyta)	chlorophyll a and c	430-450[1]	660-680[3]
	+ carotenoids (β)	450[1] 525-30[3]	
	xanthophylls (fucozanthin diatoxanthin diadinoxanthin)	449[1],525-30[1]	680[3]

+ major component

* only found in Cyanophyta.

1. Mantoura and Llewellyn 1983

2. Hallegraeff 1976

3. Yentsch and Yentsch 1979.

FIGURE LEGENDS

Fig.1: Final instrument assembly: a) The complete system. b) The monochromator drive unit in position.

Fig.2: Fluorescence emission spectra for tetraphenyl - butadiene showing increased peak smoothing with scan speed 1 to 9. M = overlay of manufacturer´s spectrum for comparison.

Fig.3: Fluorescence emission spectra for Rhodamine b following instrument modifications described in the text, showing the effect of scan speed on peak intensity and position. The manufacturer´s spectra is overlayed for comparison.

Fig.4: Mixer system a, incorporating an external sturred sample reservoir and flow cell arrangement.

Fig.5: Mixer system b, incorporating a small battery driven stirrer situated directly in the sample cell.

Fig.6: Total fluorescence spectra presented as ´Pseudo 3D´ plots. a) deionised water; b) non-fluorescent particle suspension showing increase in light scattering due to particles.

Fig.7: Outline of the typical features of a square data set. Points A and A´ are an equal perpendicular distance ´a´ from the diagonal.

Fig.8: ´Pseudo 3D´ presentation of data following ´fold over´ correction of the total fluorescence spectrum of Chlorella.

Fig.9: ´Pseudo 3D´ plot of total fluorescence data for Anabaena, demonstrating the swamping effect of the secondary scatter peaks.

Fig.10: Reverse ´pseudo 3D´ plot of total fluorescence data for Anabaena.

Fig.11: Comparison of excitation spectra for Asterionella formosa using data from this study and published data.

Fig.12: Total fluorescence contour plots of the green algae: a) Chlorella, b) Dictyosphaerium, c) Pediastrum.

Fig.13: Total fluorescence contour plots of the blue - green algae: a) Anabaena, b) Aphanizomenon, c) Microcystis.

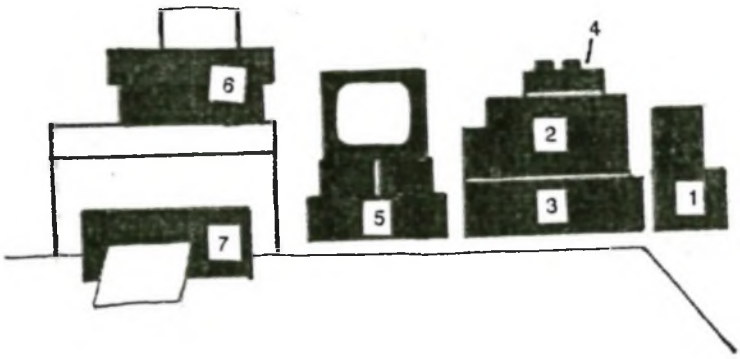
Fig.14: Total fluorescence contour plots of the diatoms: a) Asterionella, b) Fragilaria, c) Stephanodiscus.

Fig.15: Total fluorescence contour plot of the algal growth medium.

Fig.16: Positions of the most sensitive fluorescence regions for the algae under study.

Figure 1 : Final Instrument Assembly

a. The full working system

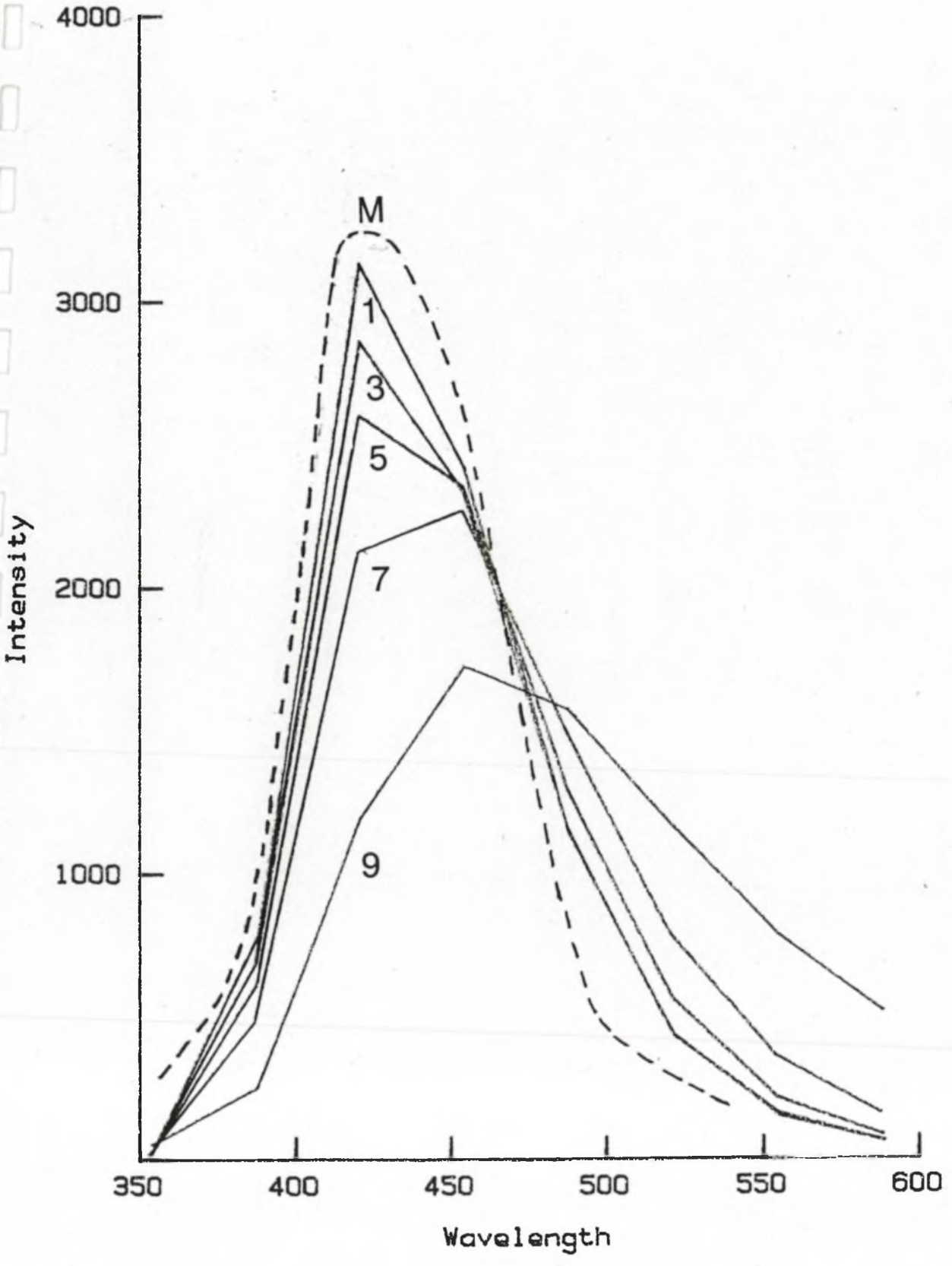


KEY:

1. Power supply units.
2. Model 204 fluorimeter.
3. FBA fluorimeter controller.
4. Monochromator drive unit.
5. Apple IIe microcomputer.
6. Printer.
7. Plotter.

b. The monochromator drive unit in position

Figure 2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2000
1000
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3
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7
9
350
400
450
500
550
600
Wavelength

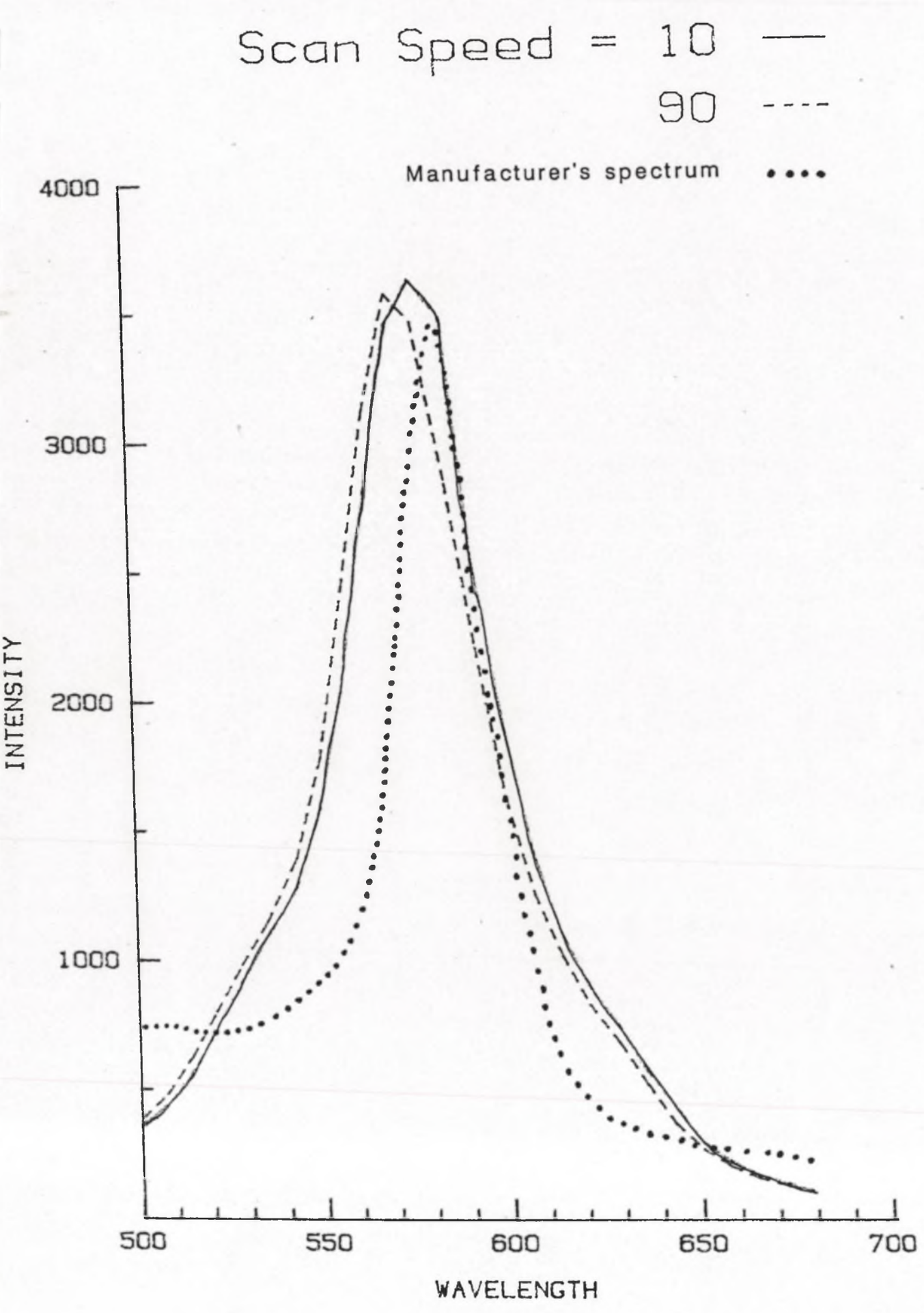

Scan Speed = 10
90
Manufacturer's spectrum
4000
3000
2000
1000
INTENSITY
500
550
600
650
700
WAVELENGTH

Figure 3

MIXER SYSTEMS

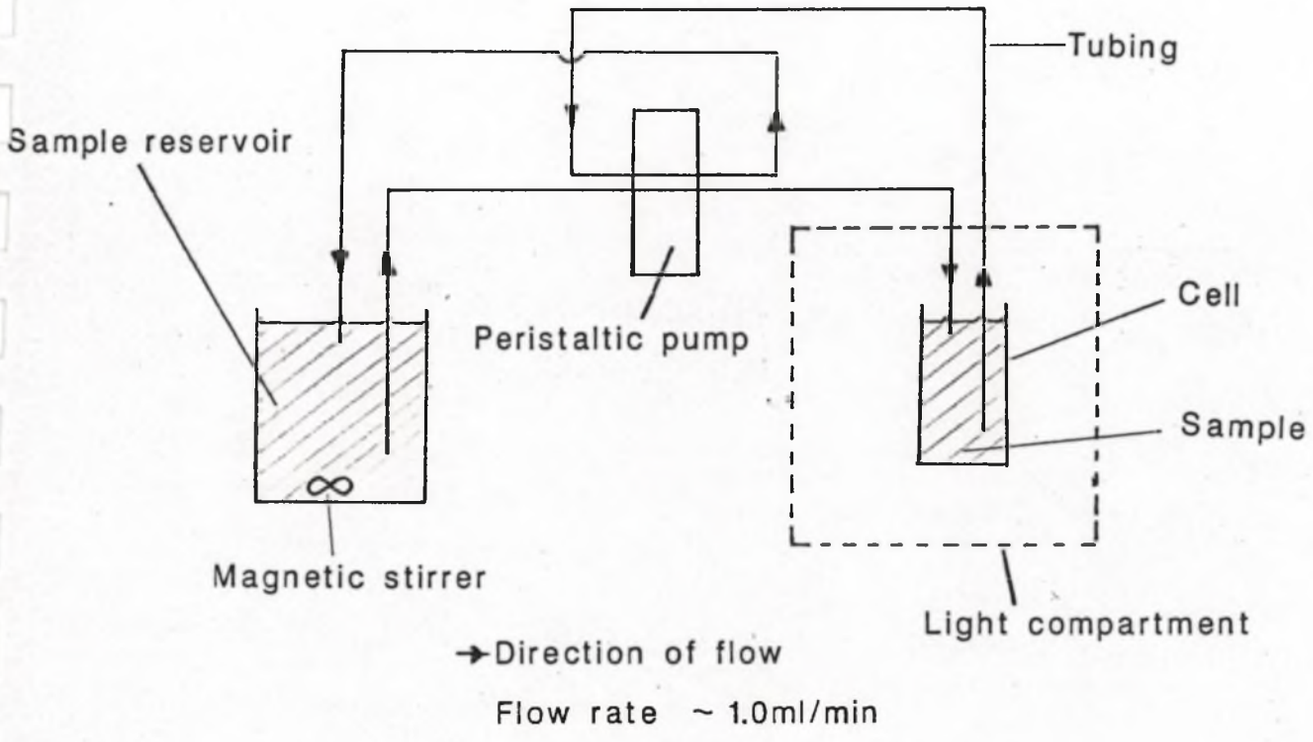


Figure 4

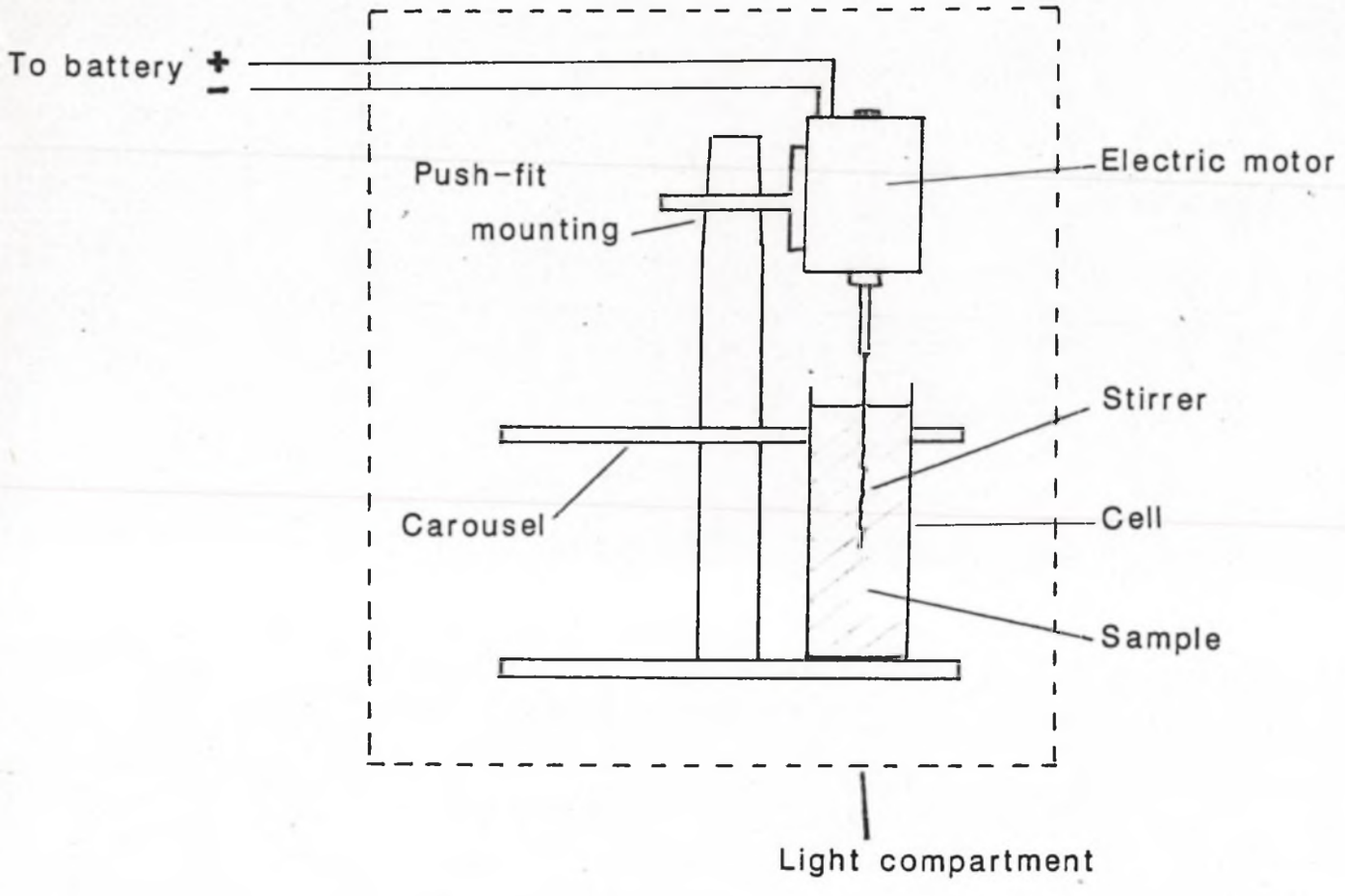
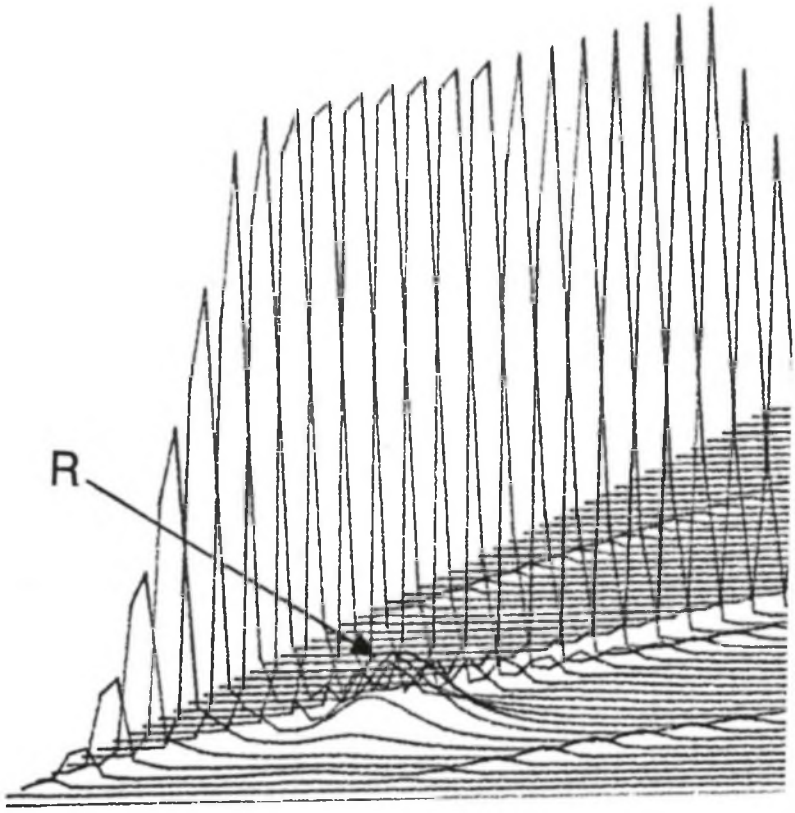


Figure 5

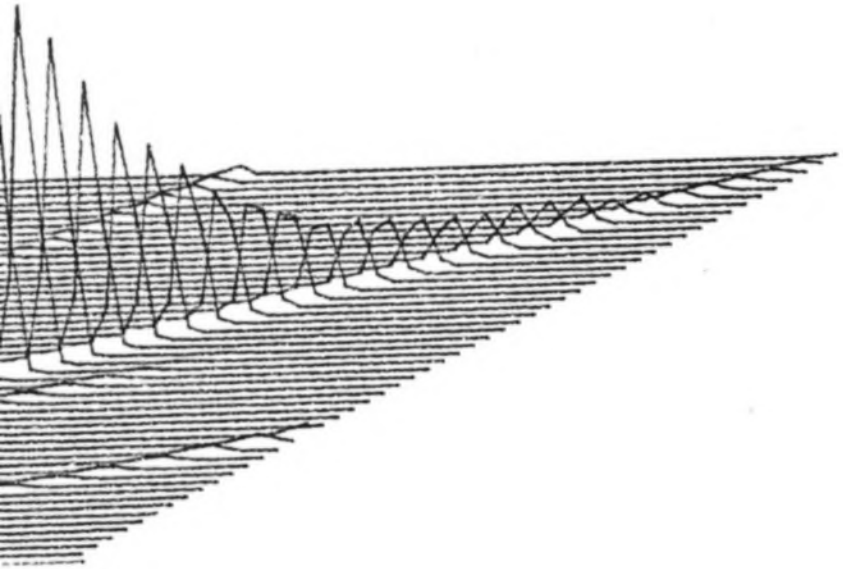
H2O BLANK 22-10-1984

Emission intensity scale factor : 1



220

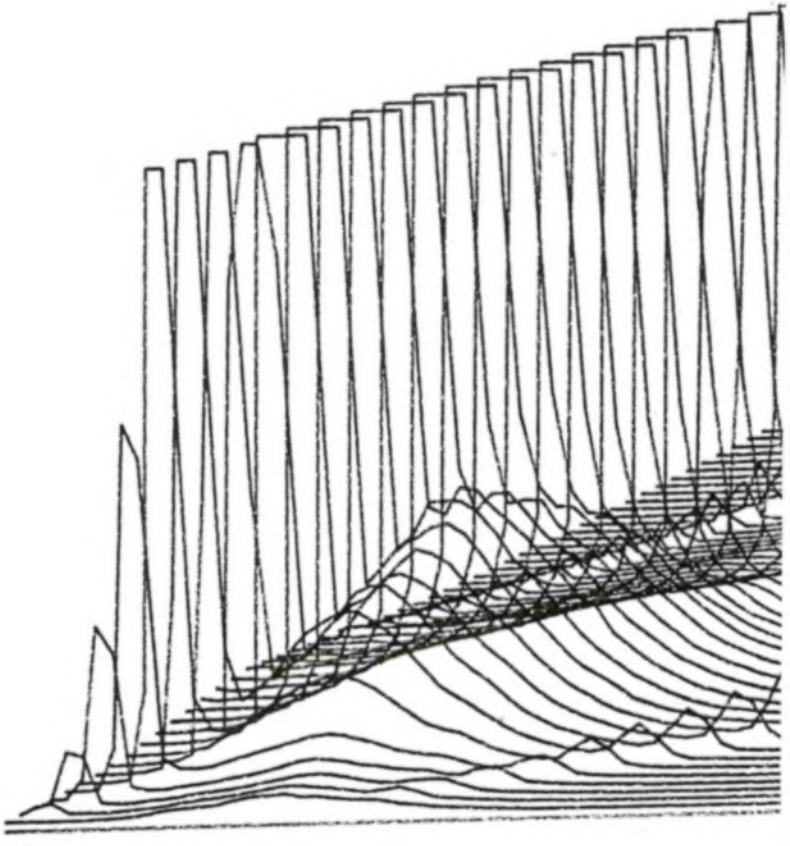
Figure 6a



780

11.0 MICRONS 1-11-1984

Emission intensity scale factor : 1



220

Figure 6b

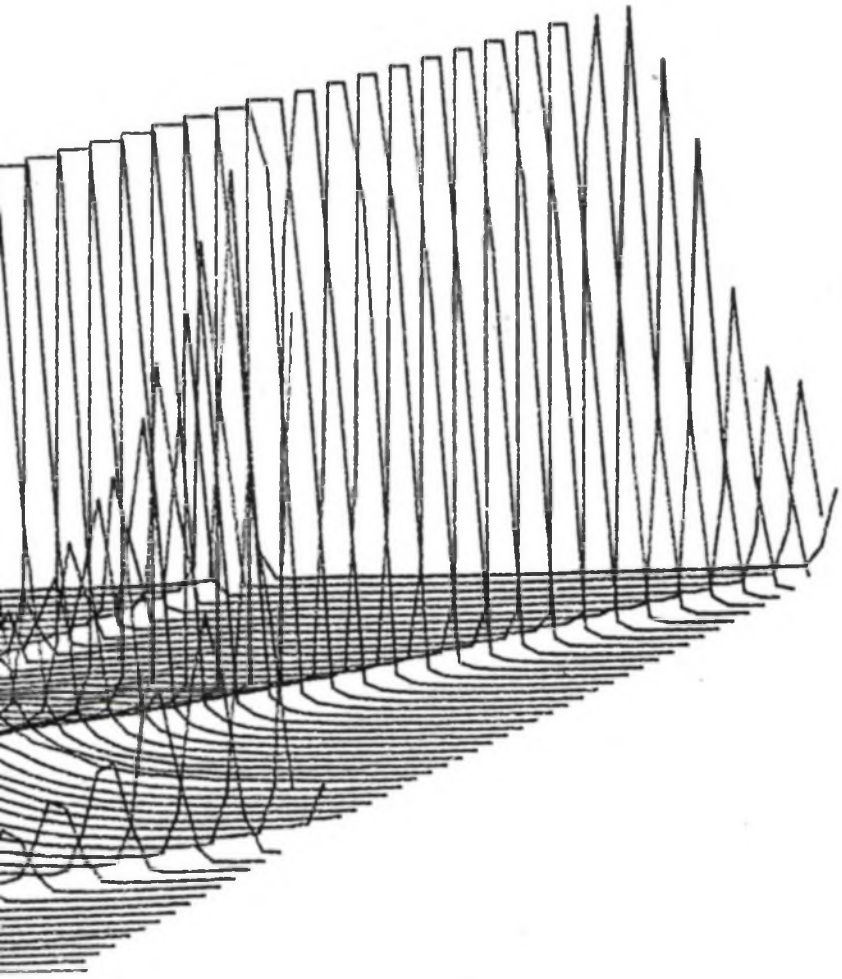
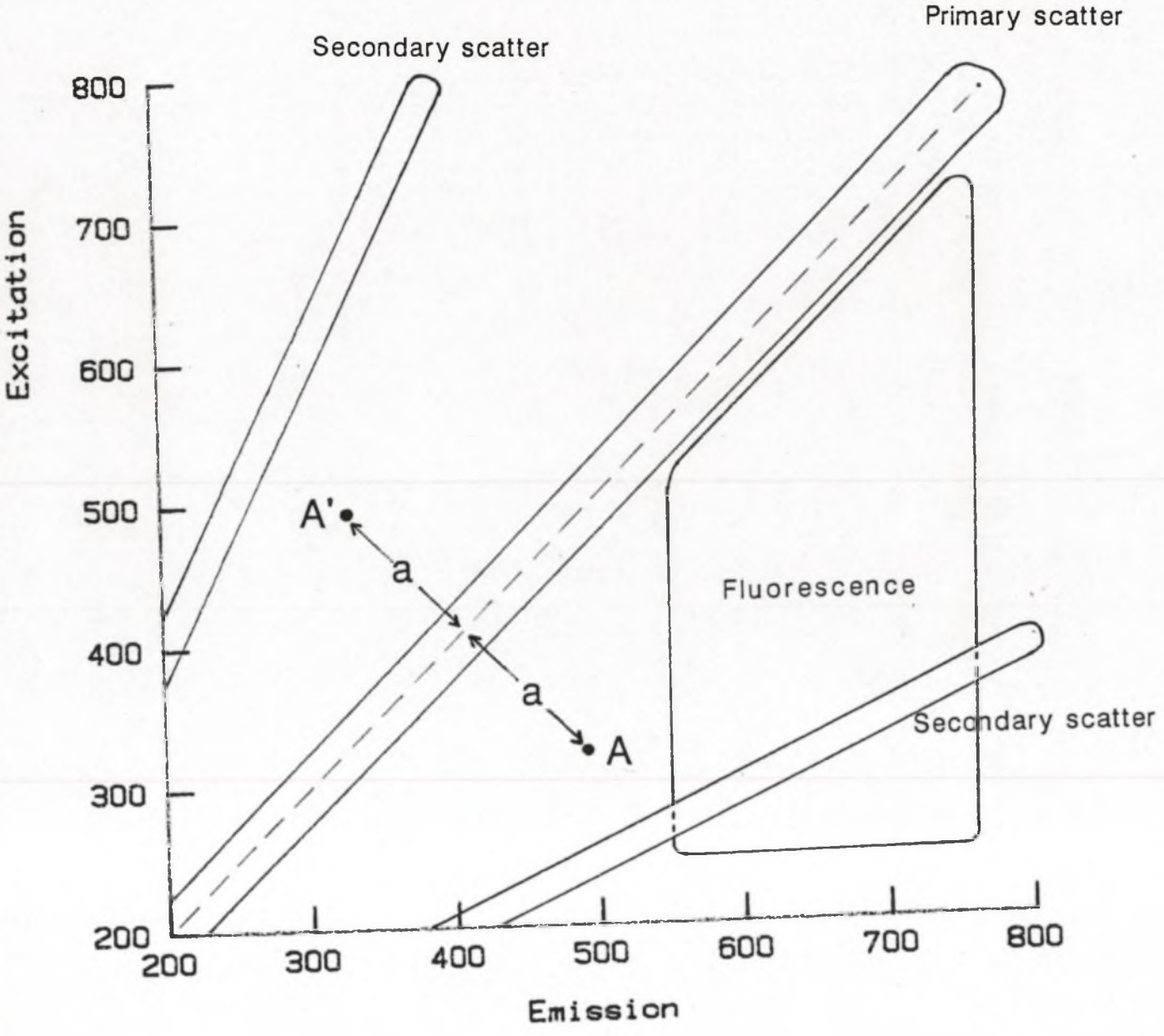


Figure 7


Primary scatter
Secondary scatter
800
700
600
500
400
300
200
Excitation
A'
a
a
A
Fluorescence
Secondary scatter
200
300
400
500
600
700
800
Emission

CHLORELLA 1 22-10-1984 (#5, CHLOR1.DATA)

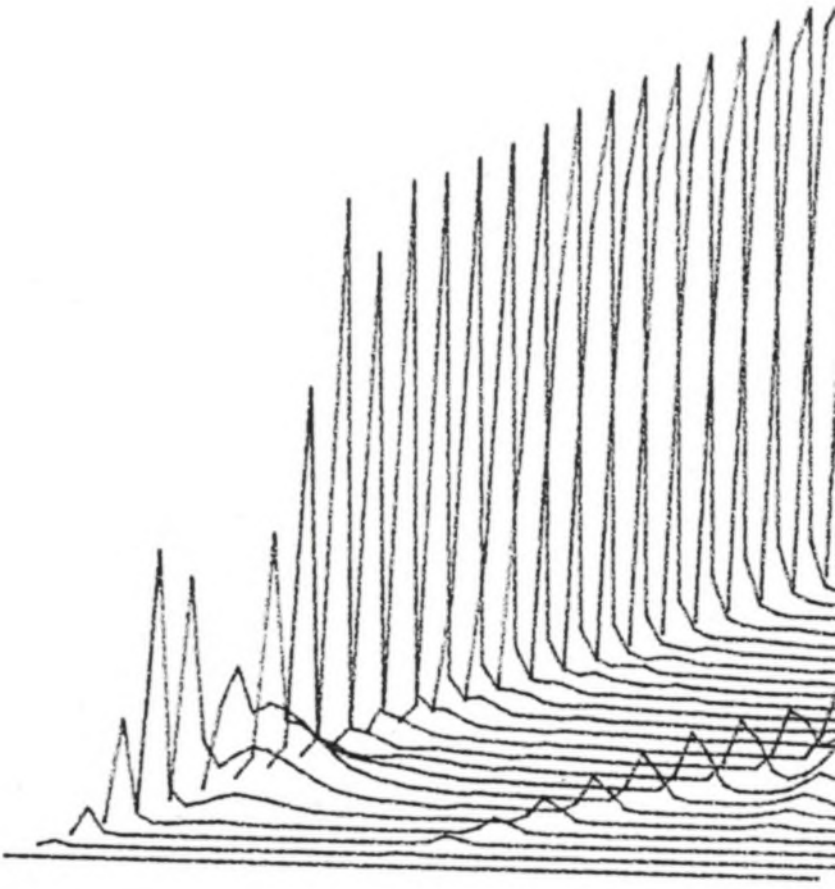
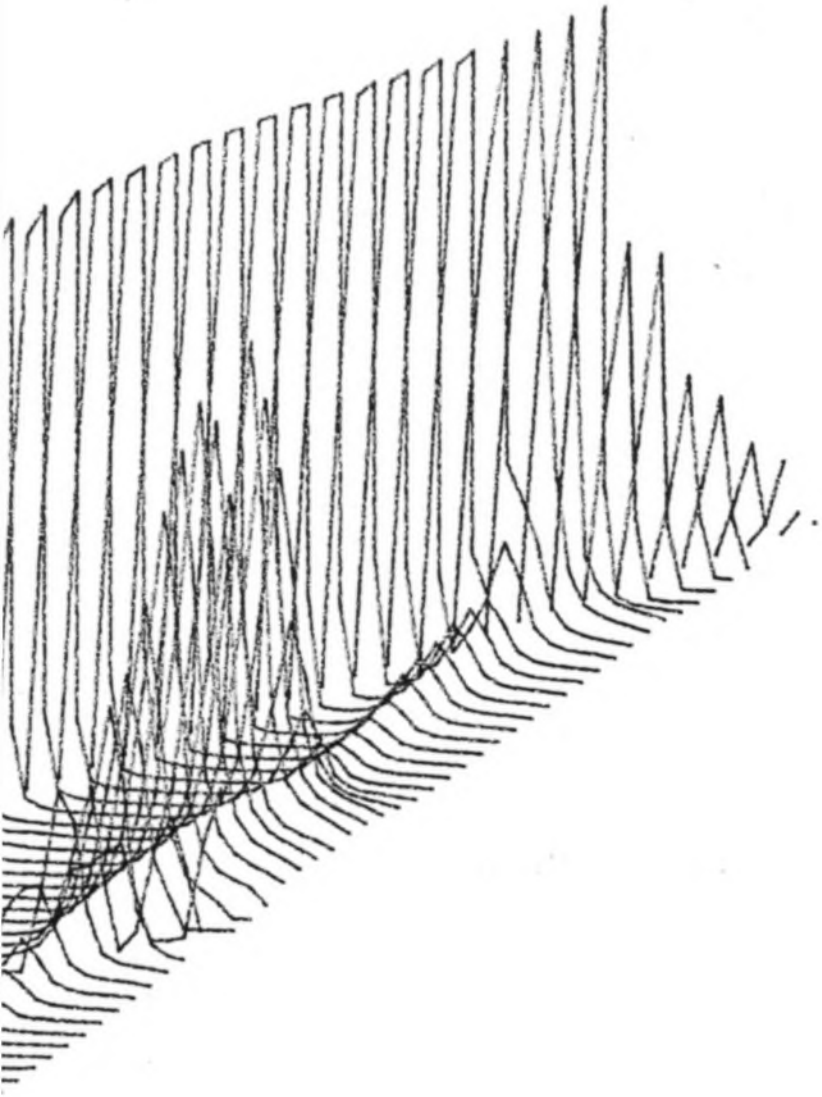


Figure 8



Anabaena L102 18-11-1985

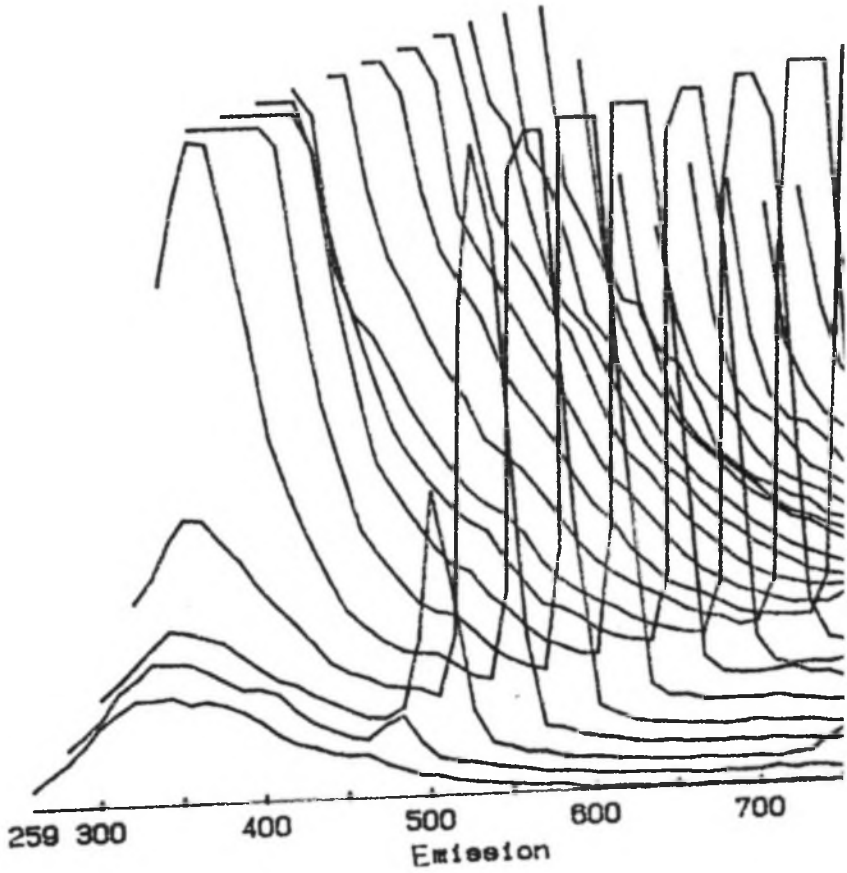
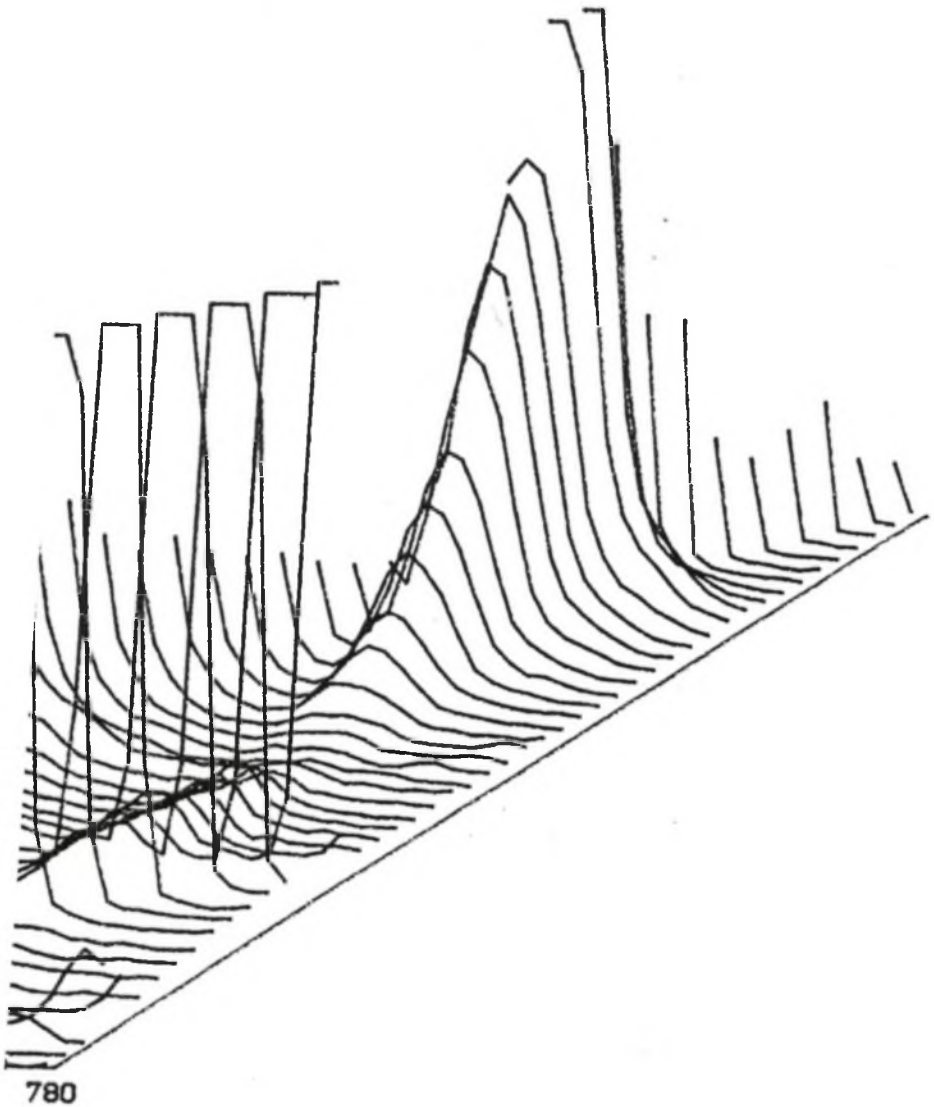


Figure 9



Anabaena L102 18-11-1985

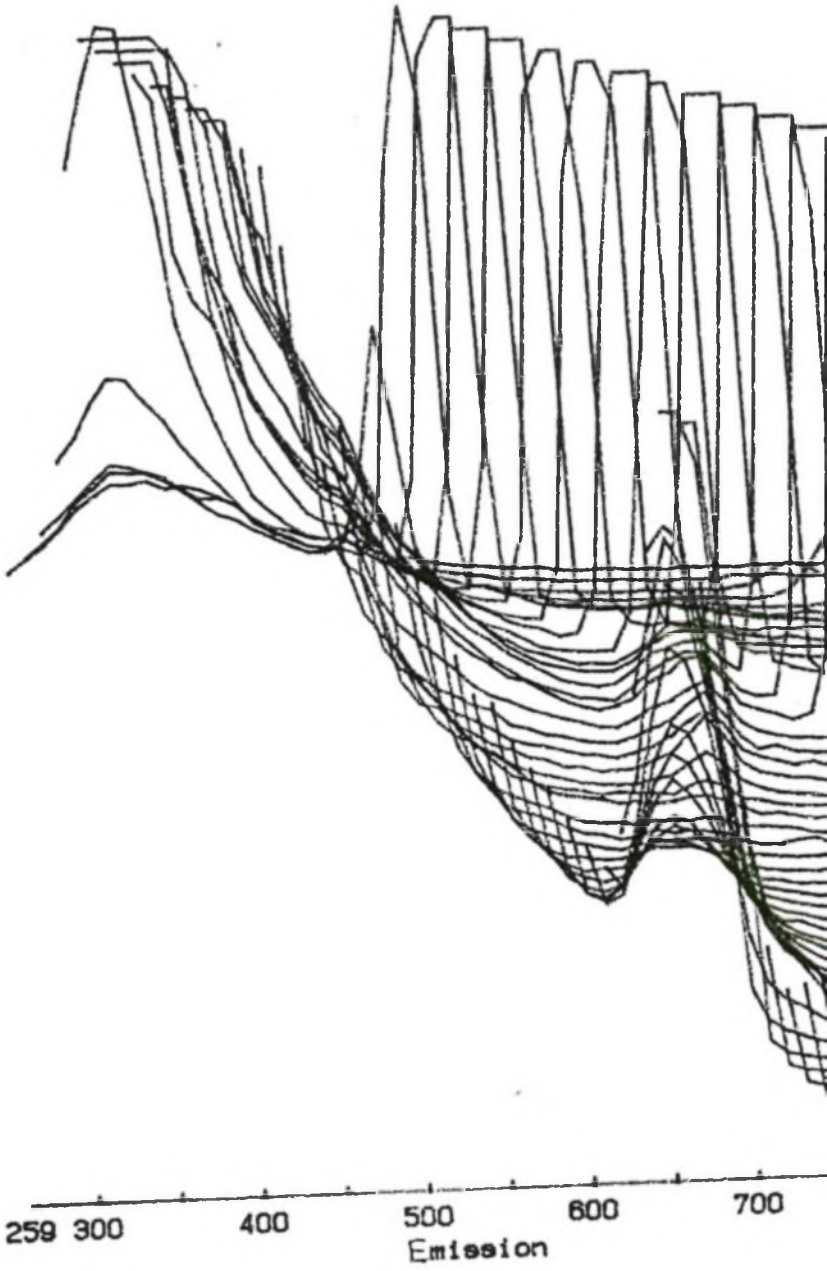
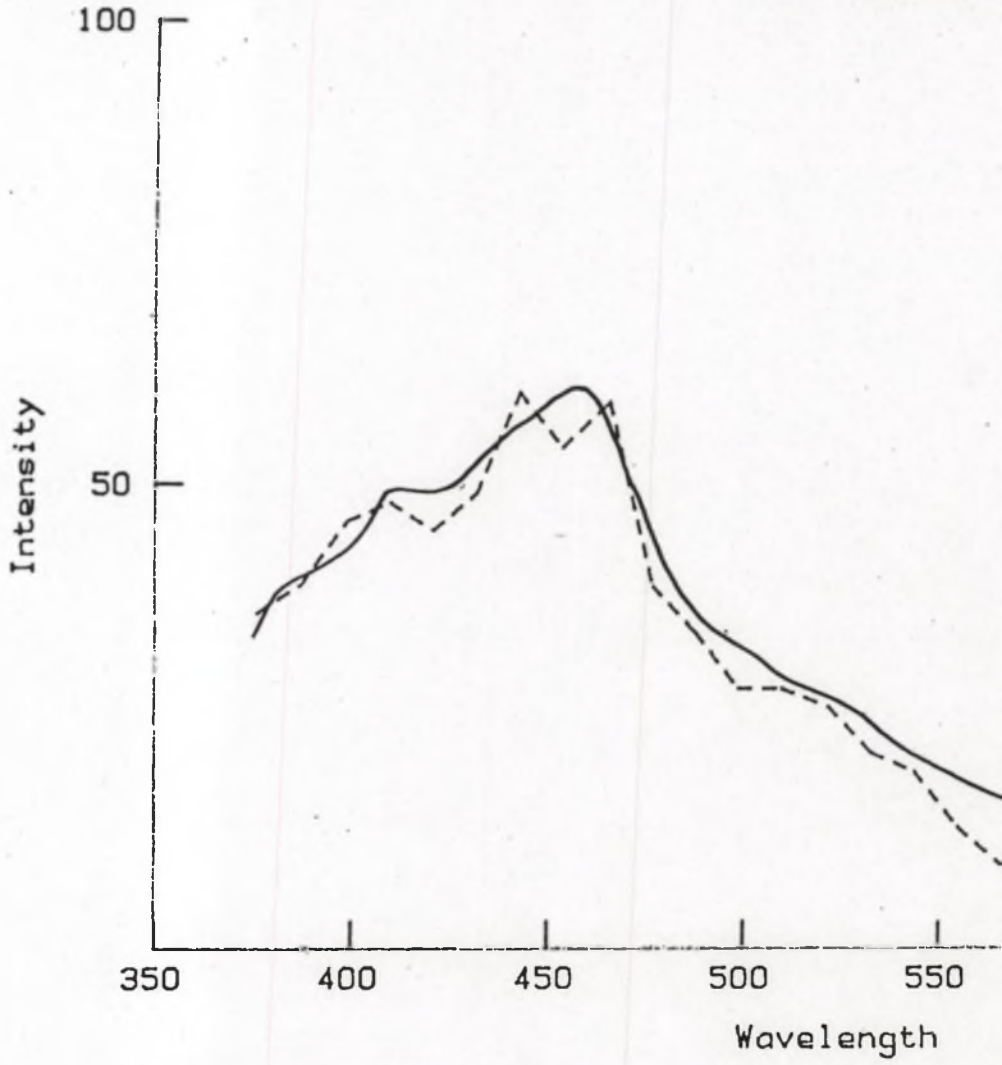
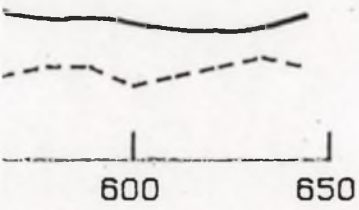


Figure 10



Excitation spectrum for Asterionella formosa : ——

Figure 11



— Heaney 1978, Em = 685nm

-- This study, Em = 682nm

Figure 12a

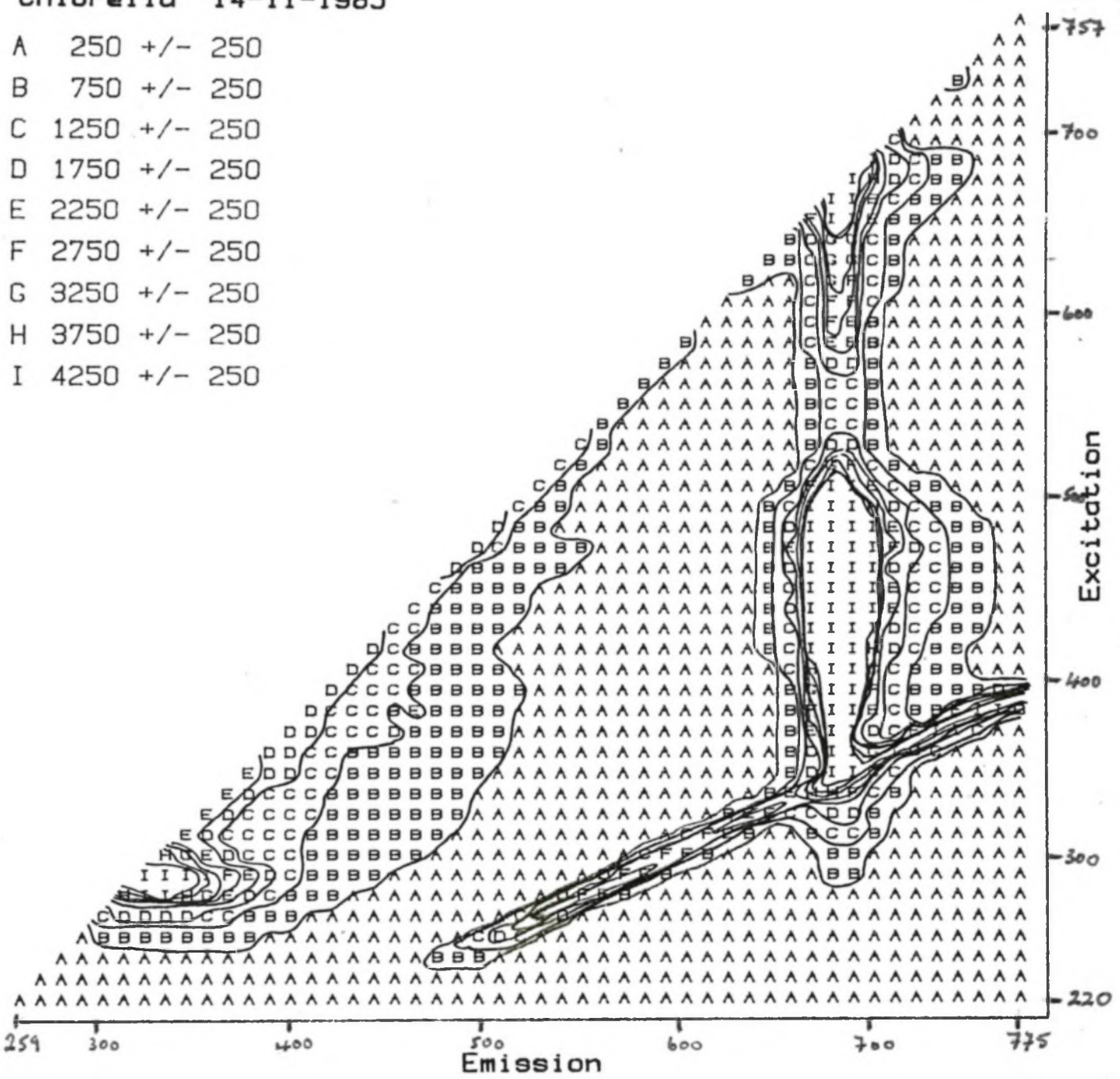

Chlorella 14-11-1985
A 250 +/- 250
B 750 +/- 250
C 1250 +/- 250
D 1750 +/- 250
E 2250 +/- 250
F 2750 +/- 250
G 3250 +/- 250
H 3750 +/- 250
I 4250 +/- 250
757
700
600
400
300
220
Excitation
259
300
400
500
600
700
775
Emission

Figure 12b

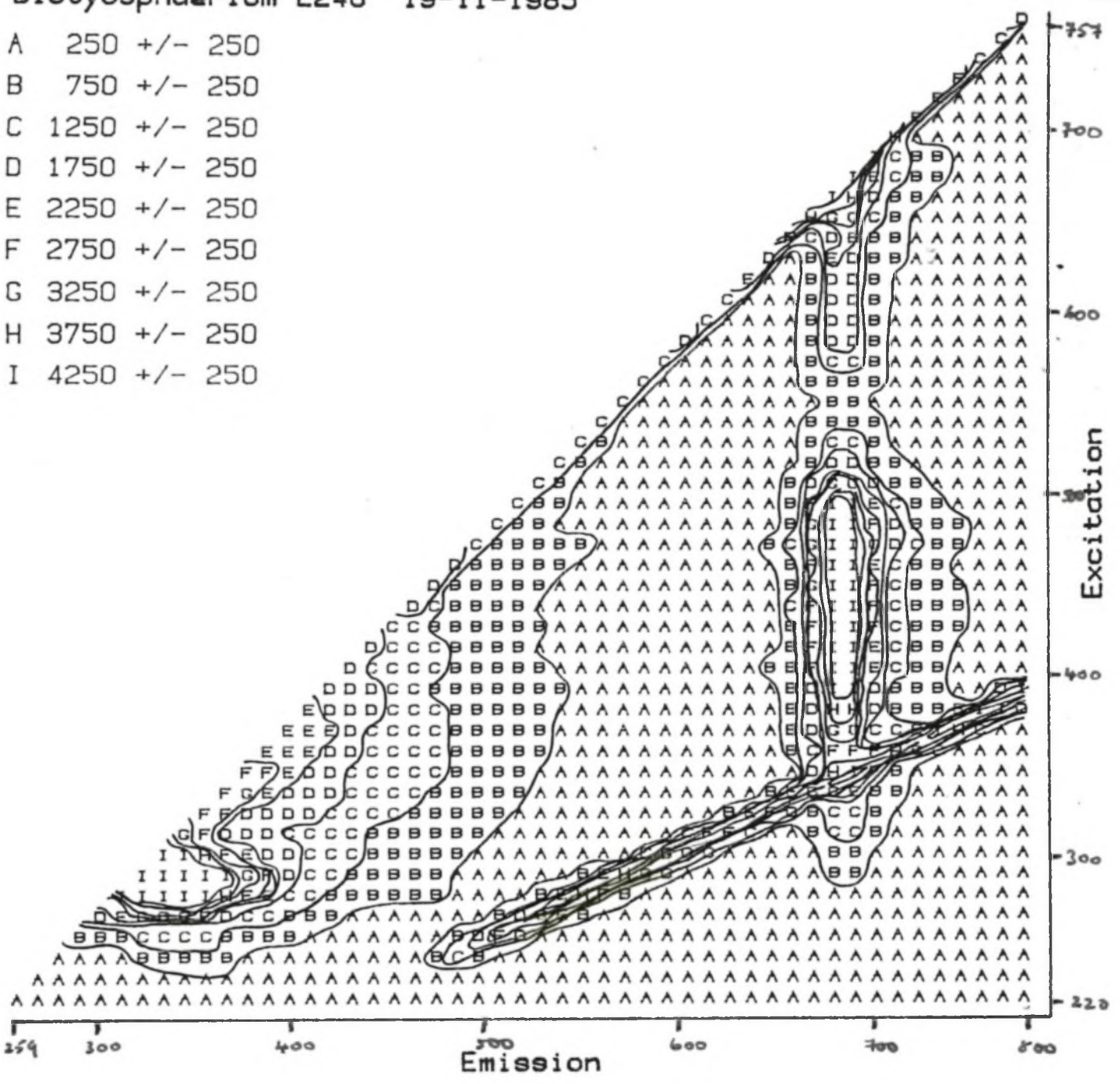

Dictyosphaerium L246 19-11-1985
A 250 +/- 250
B 750 +/- 250
C 1250 +/- 250
D 1750 +/- 250
E 2250 +/- 250
F 2750 +/- 250
G 3250 +/- 250
H 3750 +/- 250
I 4250 +/- 250
Excitation
Emission

Figure 12c

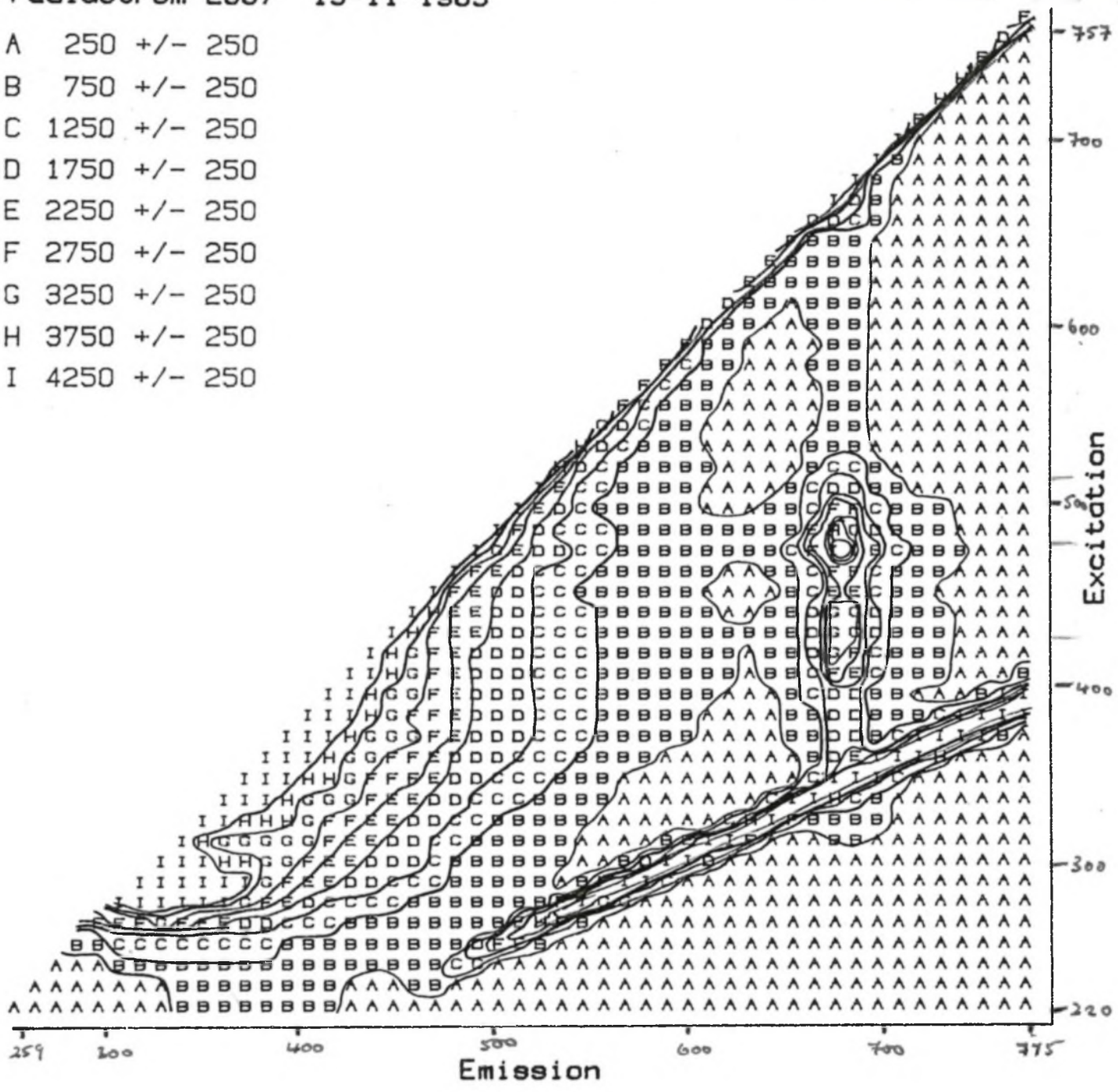

Pediastrum L387 19-11-1985
A 250 +/- 250
B 750 +/- 250
C 1250 +/- 250
D 1750 +/- 250
E 2250 +/- 250
F 2750 +/- 250
G 3250 +/- 250
H 3750 +/- 250
I 4250 +/- 250
757
700
600
400
300
220
Excitation
259
300
400
500
600
700
775
Emission

Figure 13a

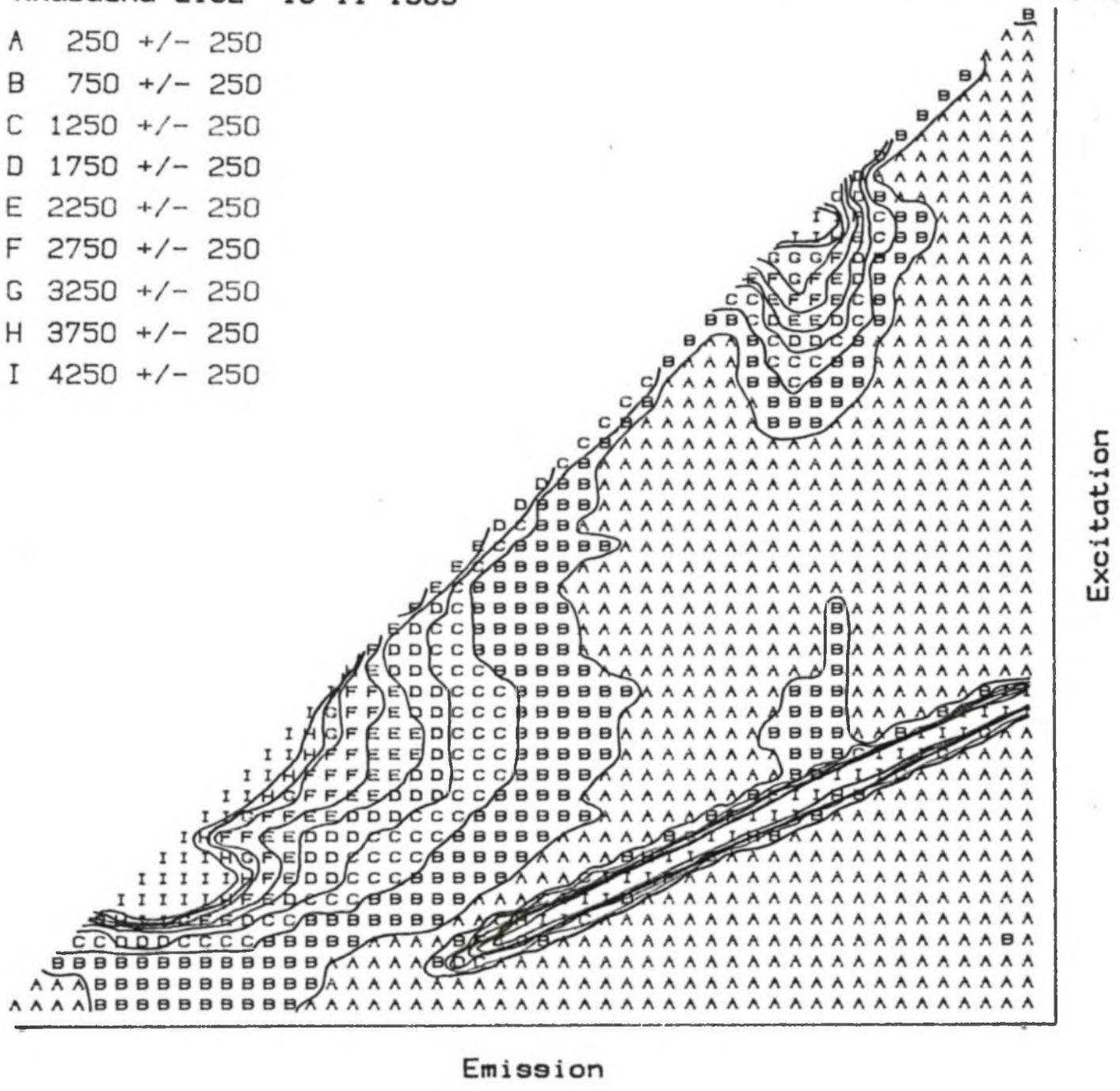

Anabaena L102 18-11-1985
A 250 +/- 250
B 750 +/- 250
C 1250 +/- 250
D 1750 +/- 250
E 2250 +/- 250
F 2750 +/- 250
G 3250 +/- 250
H 3750 +/- 250
I 4250 +/- 250
Excitation
Emission

Figure 13b

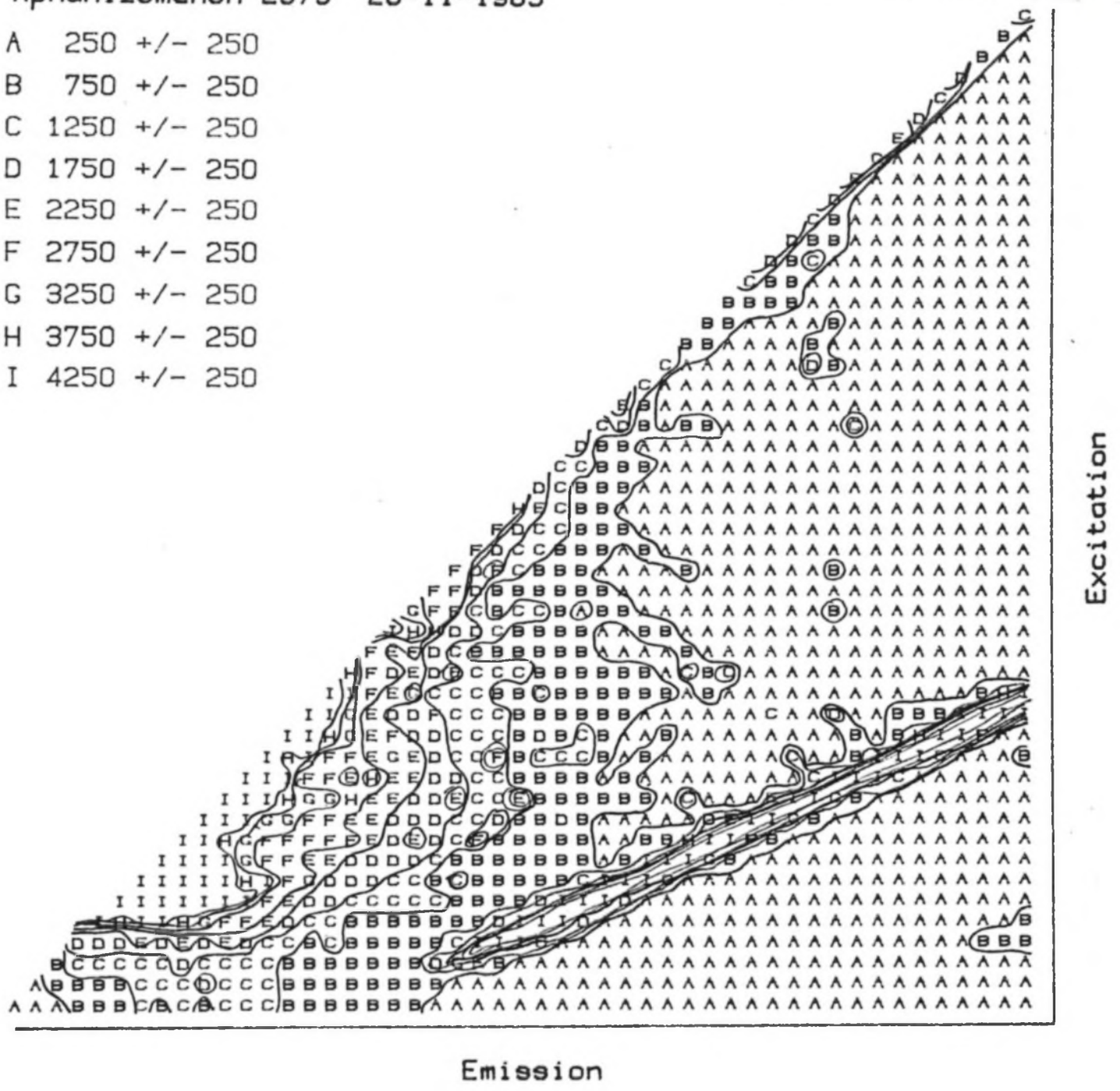

Aphanizomenon L373 20-11-1985
A 250 +/- 250
B 750 +/- 250
C 1250 +/- 250
D 1750 +/- 250
E 2250 +/- 250
F 2750 +/- 250
G 3250 +/- 250
H 3750 +/- 250
I 4250 +/- 250
Excitation
Emission

Figure 13c

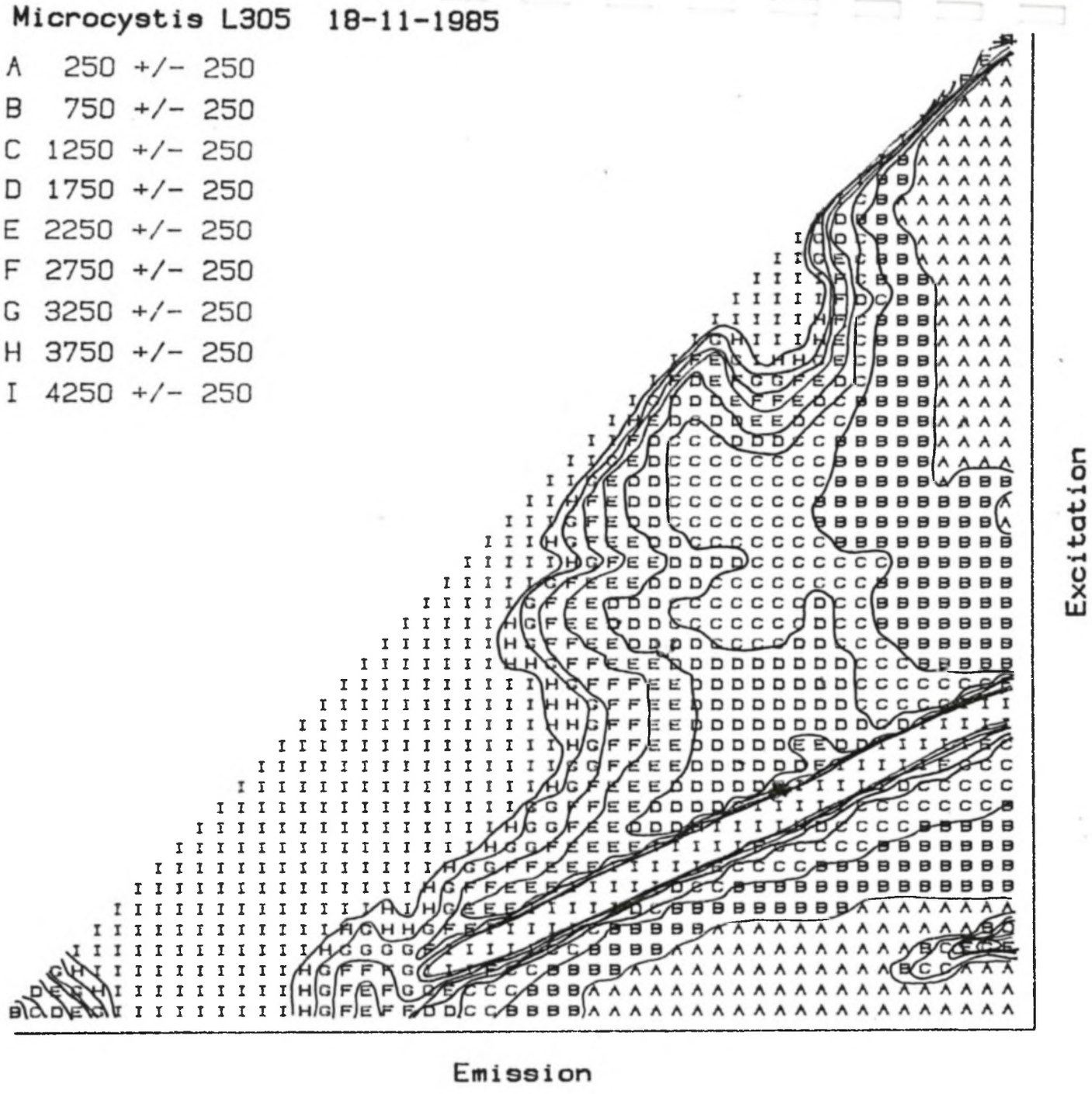

Microcystis L305 18-11-1985
A 250 +/- 250
B 750 +/- 250
C 1250 +/- 250
D 1750 +/- 250
E 2250 +/- 250
F 2750 +/- 250
G 3250 +/- 250
H 3750 +/- 250
I 4250 +/- 250
Excitation
Emission

Figure 14a

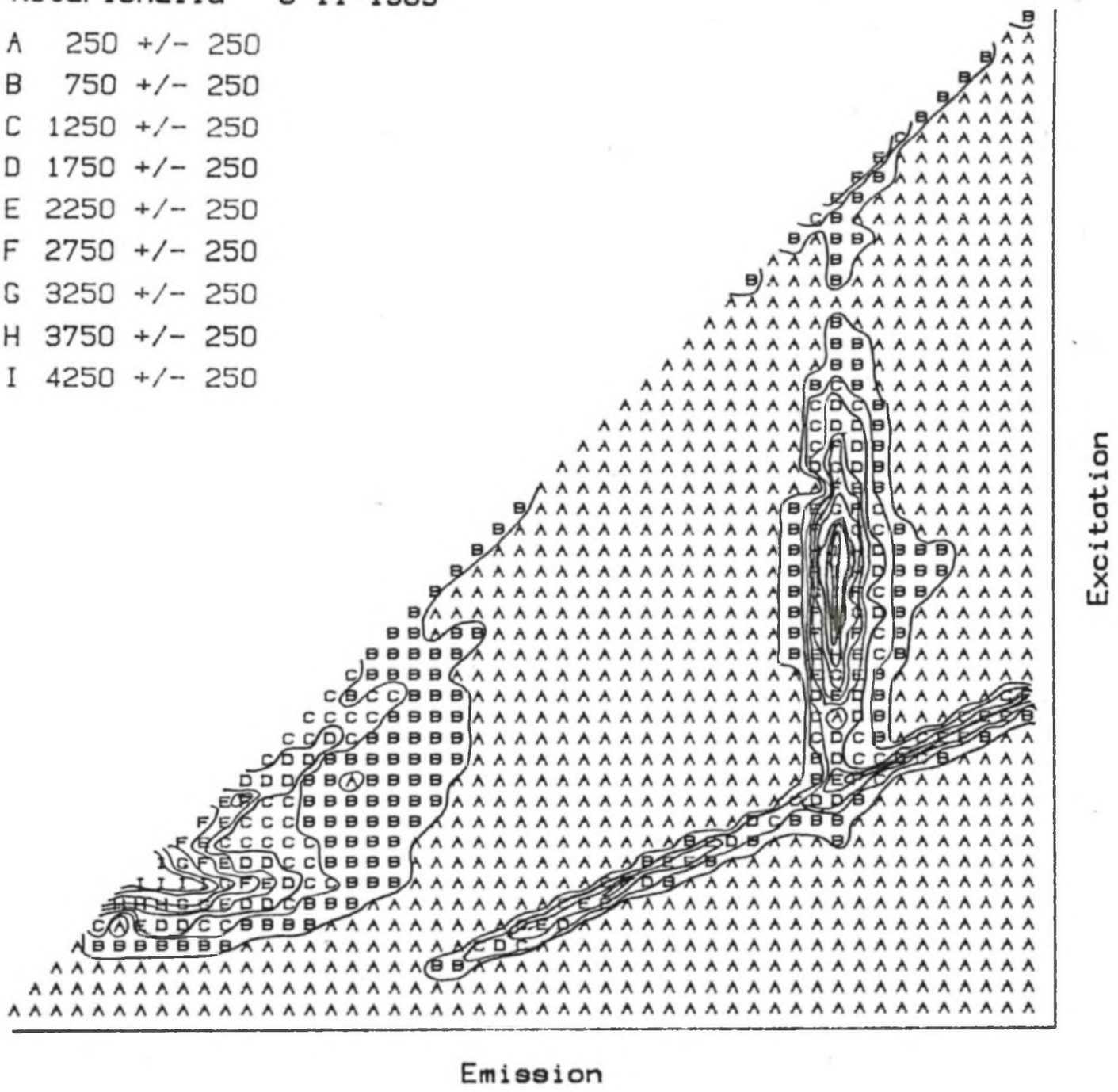

Asterionella 8-11-1985
A 250 +/- 250
B 750 +/- 250
C 1250 +/- 250
D 1750 +/- 250
E 2250 +/- 250
F 2750 +/- 250
G 3250 +/- 250
H 3750 +/- 250
I 4250 +/- 250
Excitation
Emission

Figure 14b

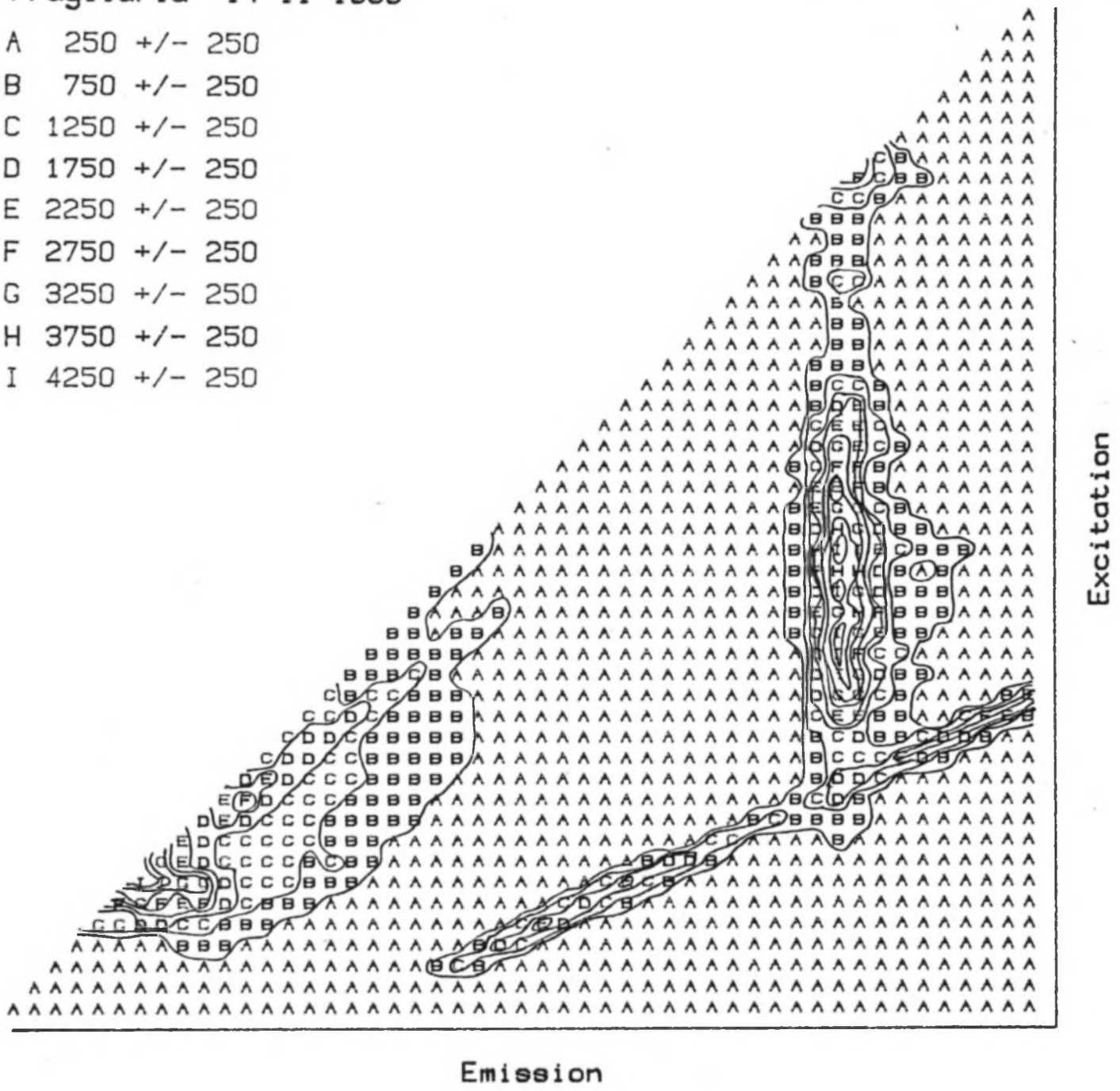

Fragilaria 14-11-1985
A 250 +/- 250
B 750 +/- 250
C 1250 +/- 250
D 1750 +/- 250
E 2250 +/- 250
F 2750 +/- 250
G 3250 +/- 250
H 3750 +/- 250
I 4250 +/- 250
Excitation
Emission

Figure 14c

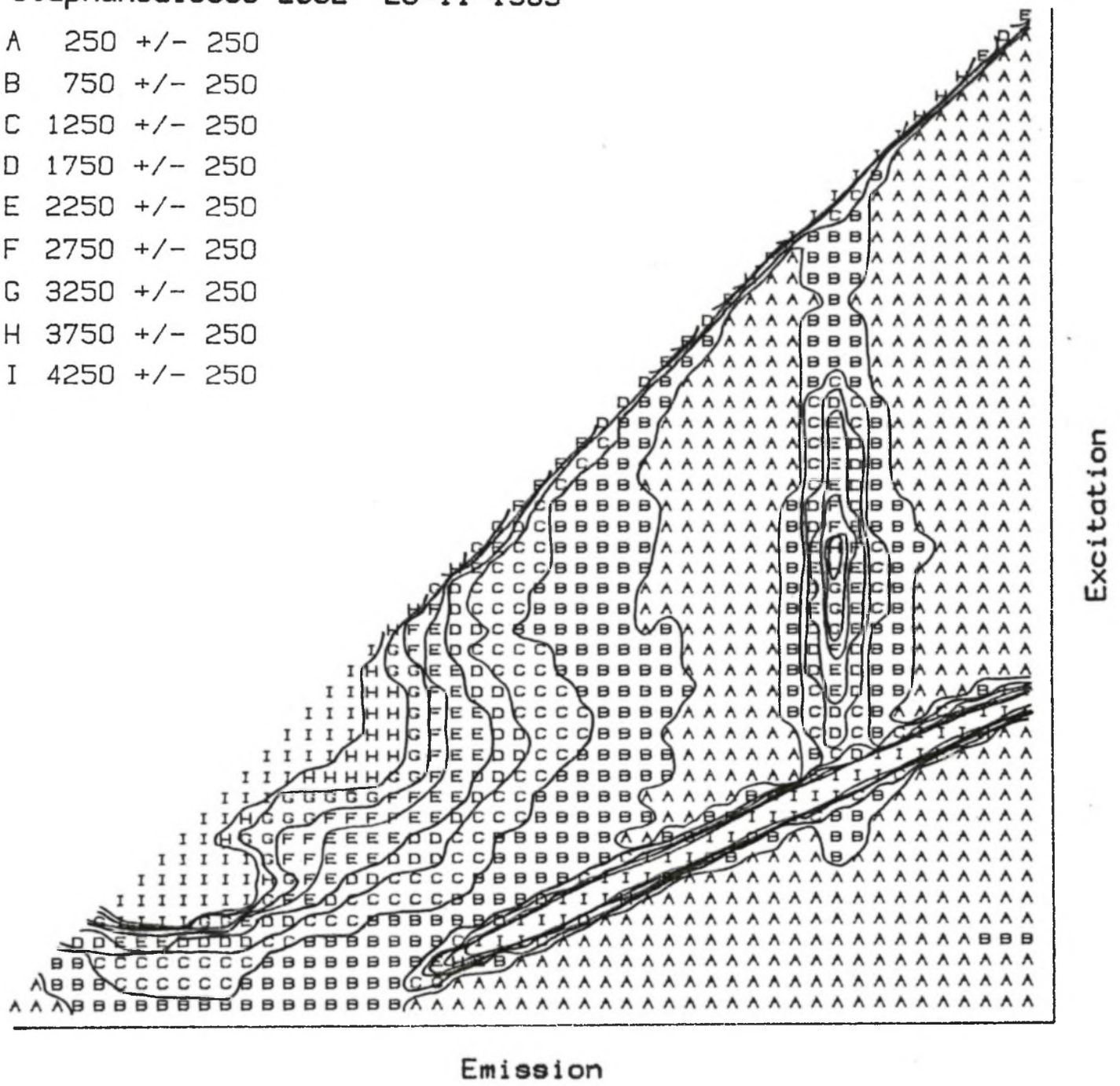

Stephanodiscus L382 20-11-1985
A 250 +/- 250
B 750 +/- 250
C 1250 +/- 250
D 1750 +/- 250
E 2250 +/- 250
F 2750 +/- 250
G 3250 +/- 250
H 3750 +/- 250
I 4250 +/- 250
Excitation
Emission

Figure 15

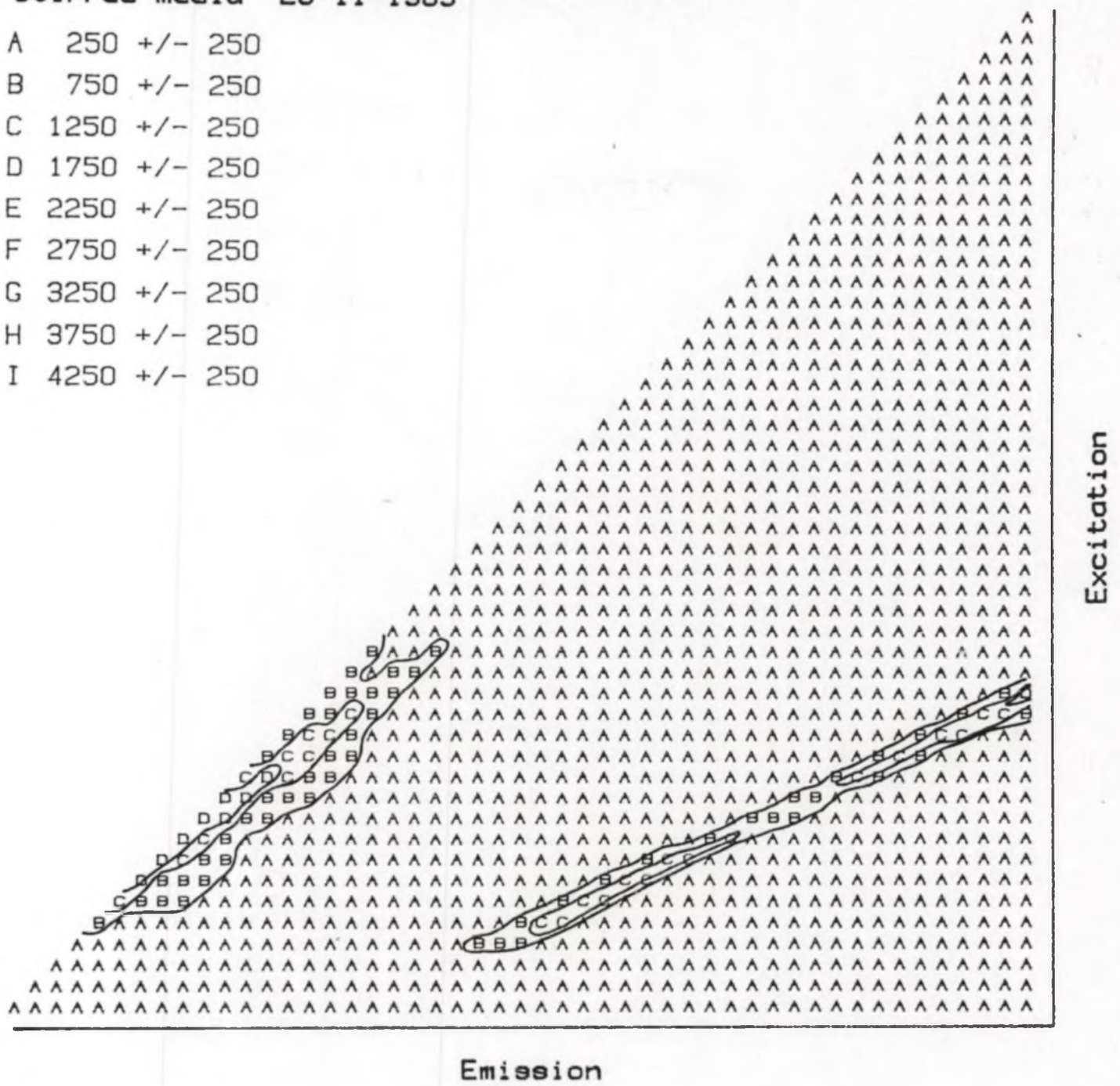
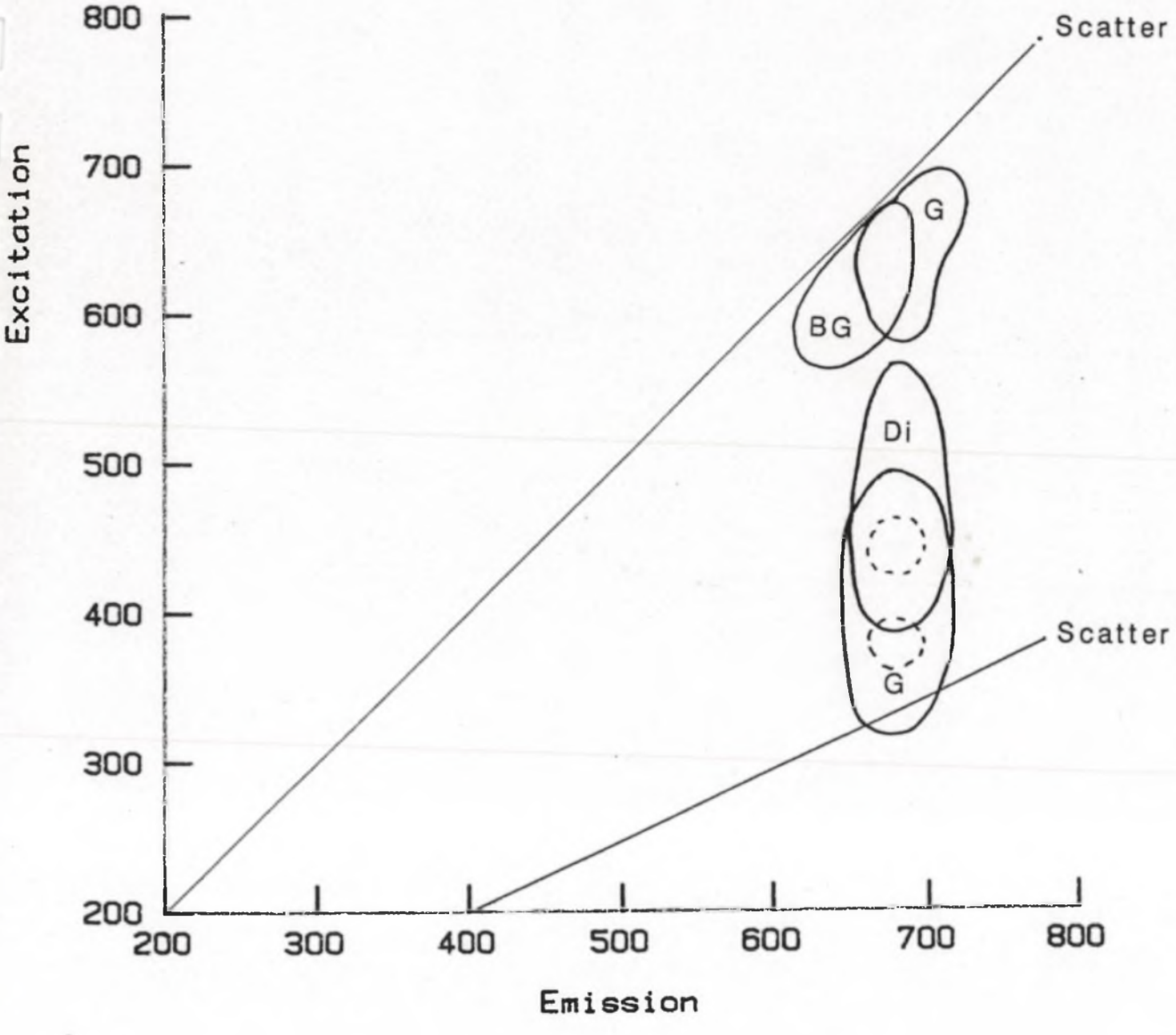

Stirred media 20-11-1985
A 250 +/- 250
B 750 +/- 250
C 1250 +/- 250
D 1750 +/- 250
E 2250 +/- 250
F 2750 +/- 250
G 3250 +/- 250
H 3750 +/- 250
I 4250 +/- 250
Excitation
Emission

Figure 16

G = Green

BG = Blue-Green

Di = Diatom



APPENDIX 1

INSTRUMENT DESIGN

Modifications to give dual scanning and automatic data collection

C.R.Cunningham & M.J.Lee

CONTENTS

MODIFICATION OF A FLUORESCENCE SPECTROMETER TO GIVE DUAL SCANNING AND AUTOMATIC DATA COLLECTION

C.R.CUNNINGHAM and M.J.LEE

January 1986

1. ABSTRACT

Many substances exhibit the property of fluorescence whereby excitation by light of a particular wavelength results in the emission of light at longer wavelengths. This characteristic may be used on suspensions of algae to determine the types of algae present by analysing the excitation and emission spectra. The normal method of doing this uses a fixed excitation wavelength and measures the emitted light over a range of wavelengths to produce emission spectra. This project aims to increase the precision of the estimation of algal content of water samples by scanning both excitation and emission so that a two-dimensional data set is obtained, which may be used as a 'finger print' for the sample.

A standard laboratory fluorescence spectrometer consists of a white light source (usually a xenon discharge lamp) with a monochromator to select the excitation wavelength. Fluorescence emitted by the sample is analysed by a second monochromator equipped with a photomultiplier detector. It is usually only possible to scan the excitation wavelength or the emission wavelength, but not both. This report describes the modification of such an instrument to enable both excitation and emission wavelengths to be scanned over a predetermined range, and the results stored in memory before being transfered to a microcomputer. This microcomputer stores the matrix of data on disk, and produces two dimensional contour map on a plotter, which can be used to estimates the types of algae present in the population.

This work was carried out as part of a project funded by the Water Research Centre, to whom we give thanks. Most of the hardware development was done by Mike Lee, as part of his sandwich course training. Most of the software development was done by Colin Cunningham.

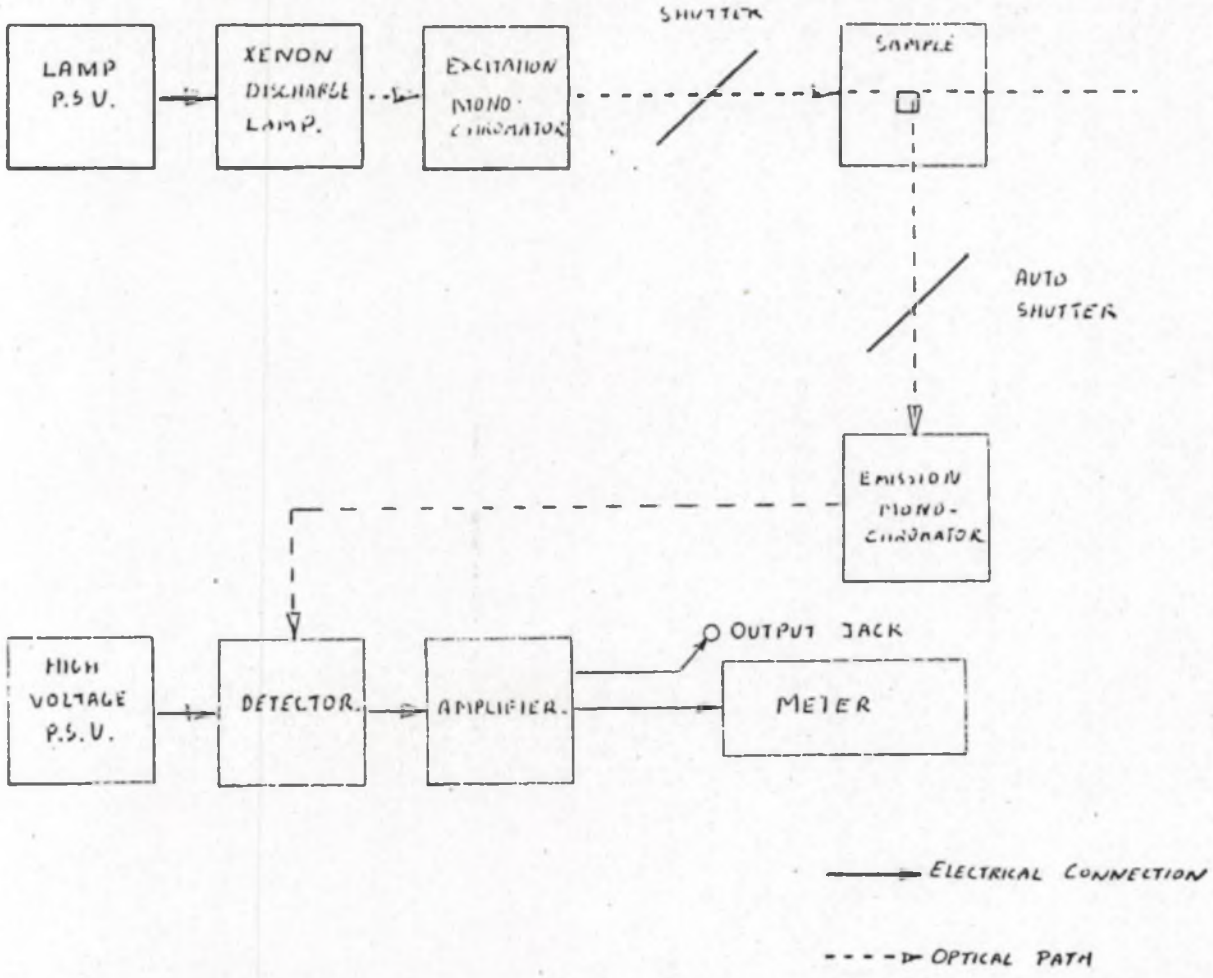
2. INTRODUCTION

The two common modes of operation of the fluorescence spectrophotometer are as follows:

1. Using a fixed excitation wavelength the detector monochromator is scanned to give an emission spectrum.

2. With the detector monochromator set at some optimum wavelength the excitation monochromator is scanned. This gives rise to an excitation spectrum. Additionally both monochromators may be scanned simultaneously at the same rate with a fixed wavelength separation between the two. This technique is known as

FIG 1 FUNCTIONAL DIAGRAM OF THE FLUORIMETER.



"synchronous scanning" and various wavelength separations may be used to obtain maximum information. Whilst the results of synchronous scanning are difficult to interpret theoretically, they can enable very sensitive comparisons to be made between samples.

The results of any of the above methods gives rise to two variables which may be simultaneously plotted using a conventional chart recorder. The object of this project was to enable both excitation and emission monochromators to be independantly driven so that the information from both 1. and 2. above is collected, and stored in memory , for a complete range of wavelengths.

The instrument available was a Perkin-Elmer 204 fluorescence spectrophotometer (Figure 1). This covers a range of 220 - 780 nM on both excitation and emission monochromators. It has an internal motor drive to scan either monochromator and the output may be selected from either an internal analogue meter, a chart recorder (0 to 10mV), or an analogue voltage output (0 to 1 V) intended to be used in conjunction with a digital voltmeter.

In addition a Quarndon QMS 85-1185DA microcomputer was available to be used as a dedicated controller. This is a bus-based 8085 system, and as such was easily expandable and provided versatile interfacing facilities.

3. HARDWARE

The general hardware requirements are outlined below:

1. A microcomputer to control the operation of the other elements.

2. A means of application of a driving force to the monochromators.

3. A means of converting the analogue output into a representative digital format.

4. A means of temporarily storing the acquired data.

5. An interface to the Apple computer, so that data may be stored permanently for plotting and manipulation. This also serves as a human interface to control the operation of the instrument.

Also, it was important that the modifications to the instrument itself were kept to a minimum so that it could be used as a conventional fluorimeter.

3.1 MICROCOMPUTER

The microcomputer available was a Quarndon QMS 85-1185DA processor board complete with a 19" rack and power supply with a

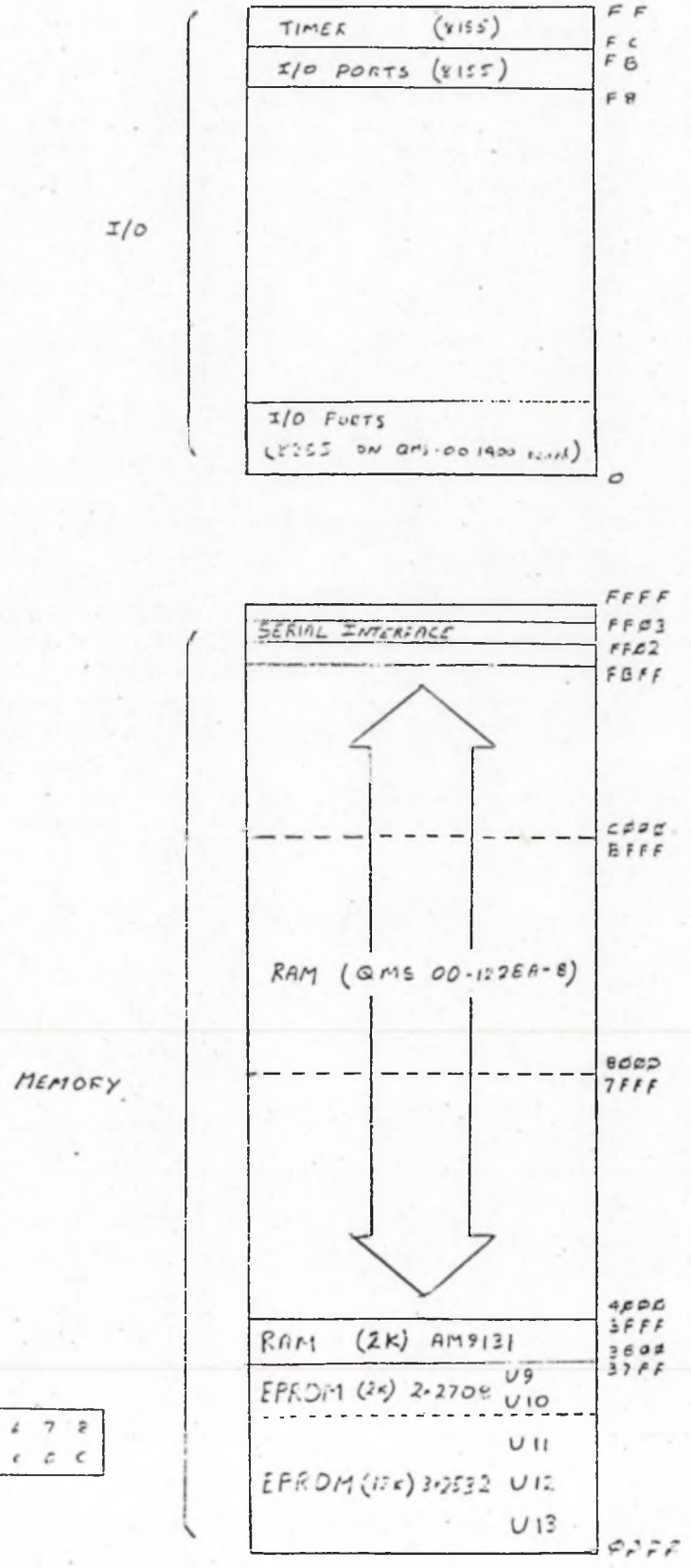


FIG. 2 QMS 82-1155 MODIFIED MEMORY MAP

six slot motherboard thus enabling additional boards to be added to the Quarndon Q-BUS system. The cpu board itself contains an AMD8085 cpu together with 2K bytes of RAM, 4K bytes of EPROM containing the monitor and assembler, a parallel interface port using an 8155 chip, and an RS232 serial interface using a 6850 UART. The system is expandable with extra boards supplied by the manufacturer. A 64Kbyte dynamic RAM card (QMS 00-1228A-8) was also installed. The memory map of the system was modified to provide 50,175 bytes of RAM between 3800H and FBFFH, and up to 14,335 bytes of EPROM between 0000H and 37FFH (in the form of 3 2532s and 2 2708s), as shown in Figure 2. The 8155 chip has 256 bytes of RAM between FE00H and FEFFH. This area was used for the Pascal stack, by initialising the stack pointer to FEFFH. All additional circuitry was mounted on a blank matrix board supplied by Quarndon for development purposes. The power supply, which is mounted within the rack itself, supplies four voltage rails (+12, +5, -5, & -12 V) thus no additional sources were required. Details of the Quarndon boards can be seen in appendix A.

3.2 MONOCHROMATOR DRIVES

The instrument itself contains a driving motor and gear mechanism which enables either monochromator to be selected and automatically scanned. Some consideration was given to utilising this feature in the modification in order to prevent the need for additional driving motors. However several problems soon became apparent:

1. There was no obvious method of measuring the increments of rotation of the monochromators.

2. The driving speed, ie. the rate of scanning could not be easily adjusted.

3. Transfering the drive between the two monochromators could prove to be mechanically difficult.

For the above reasons it was decided that the use of the internal drive motor was not practical, so an auxiliary drive was required. There were two options open to implement this: either a servo control; or stepping motors. Stepping motors were finally chosen for the following reasons:

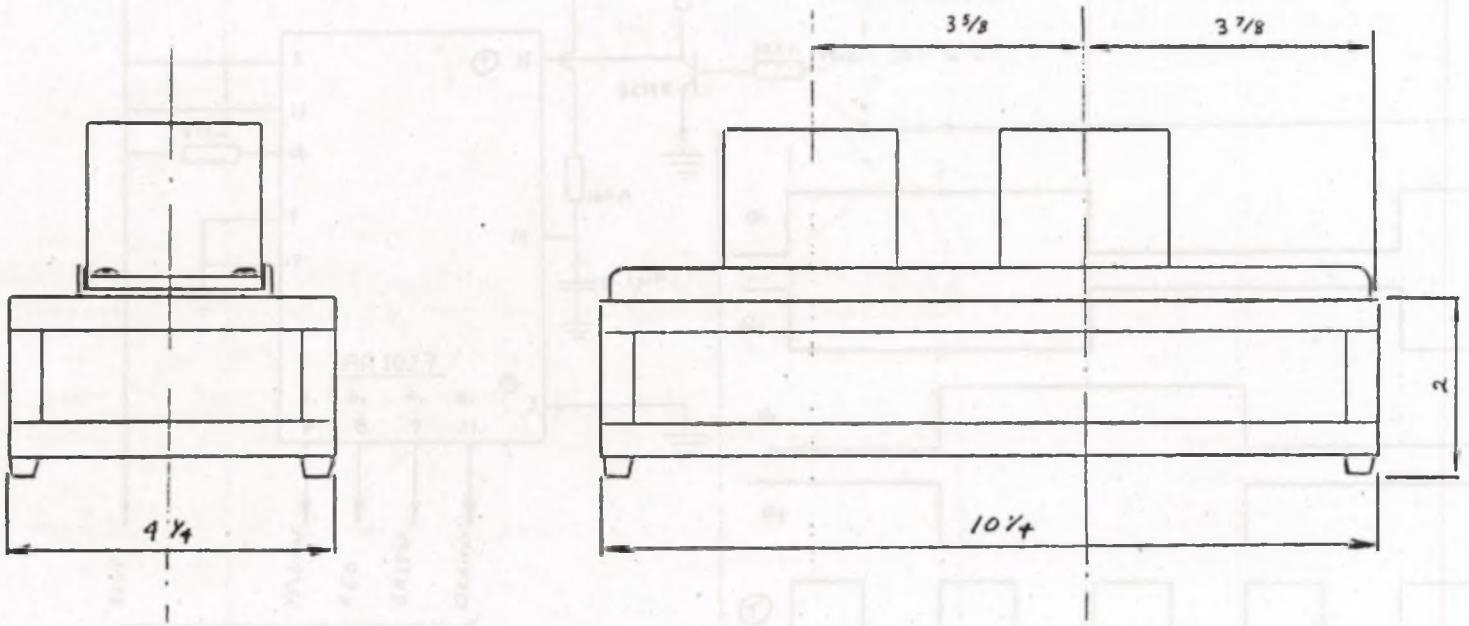
1. Ease of interfacing with digital equipment.

2. Software control of speed,thus enabling simple modifications to suit the circumstances (amplifier response, multiple step increments etc.).

3. No need to measure increments of rotation since each single step results in a known angular displacement; thus only an initial datum point is required ,together with a software counter to define the shaft position at any time.

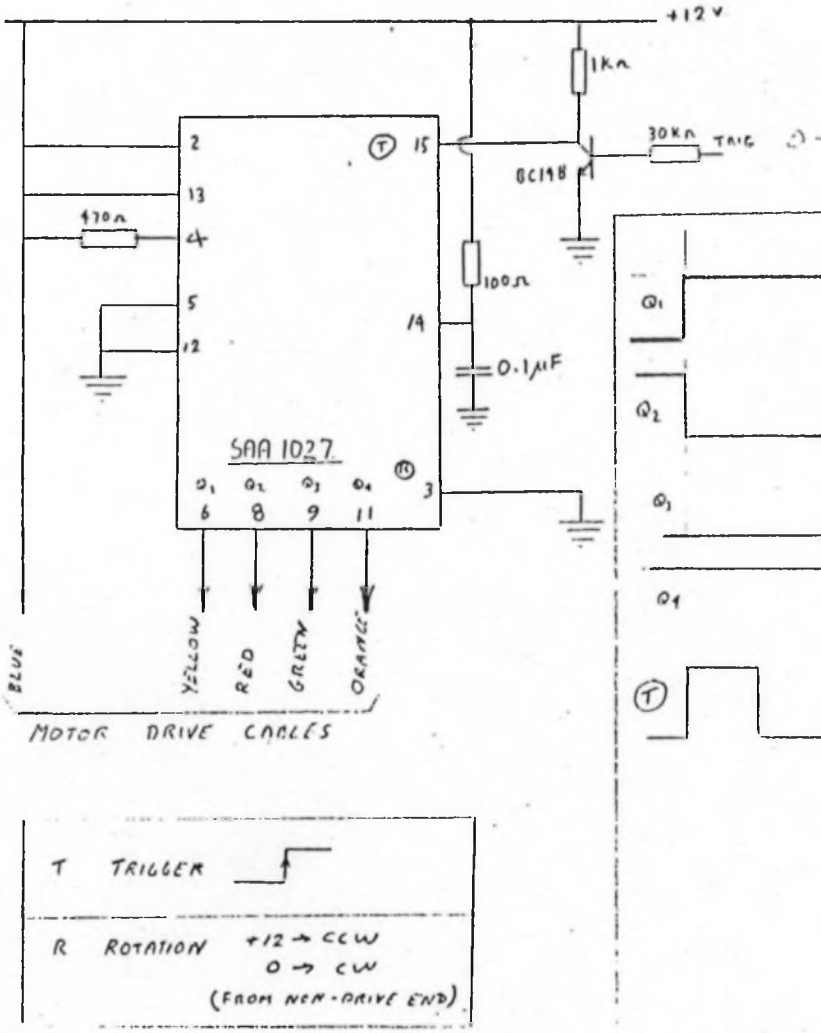
4. The motors provide sufficiently small increments (1.8^{o}) with adequate torque to obviate the need for any reduction gearing. Therefore, the motors could be mounted directly above their respective controls requiring only a simple coupling to transmit

FIG. 3 MOTOR FRAMEWORK



ALL DIMENSIONS IN INCHES.

FIG 5. DRIVE CIRCUIT & TIMING DIAGRAM



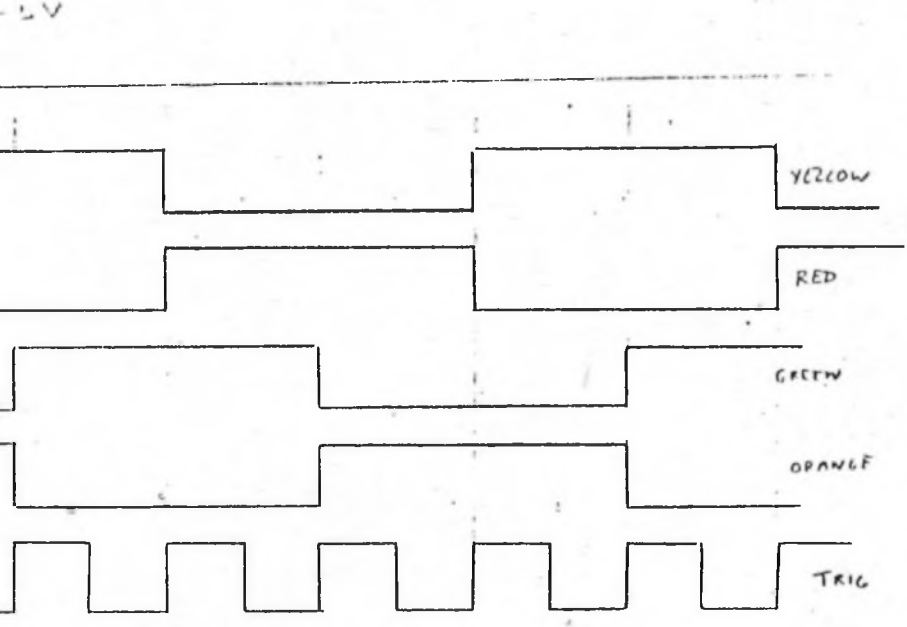
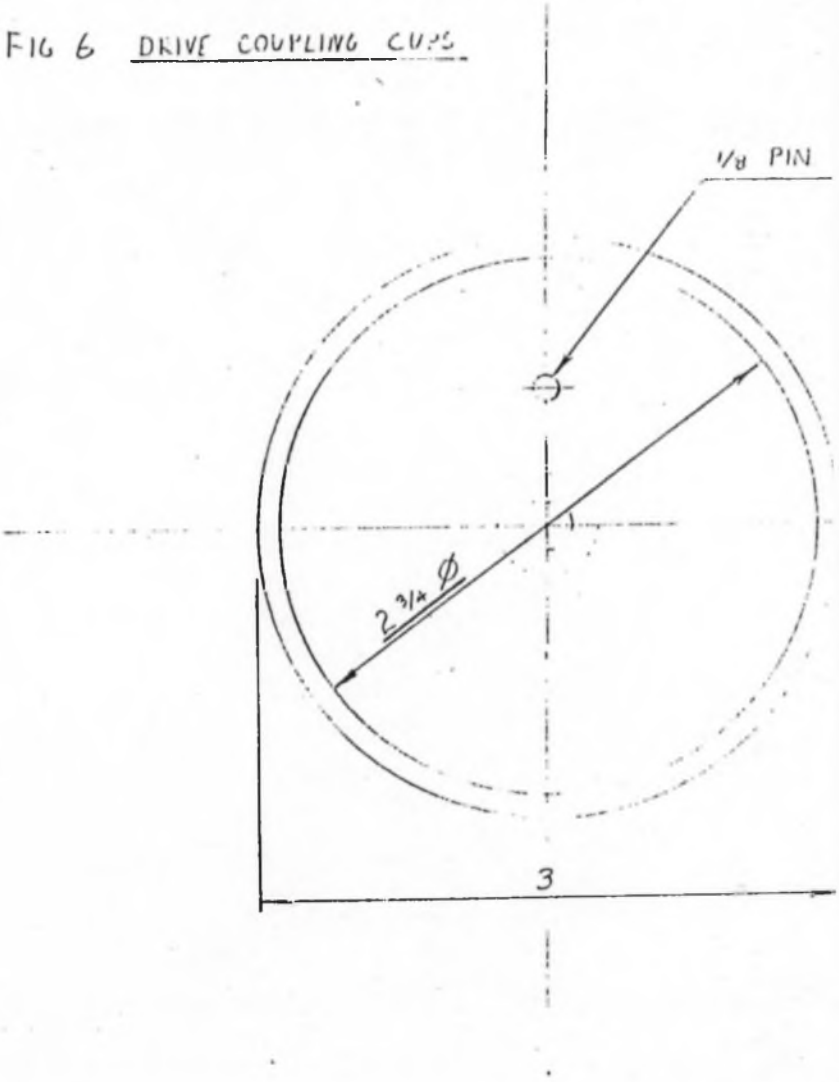
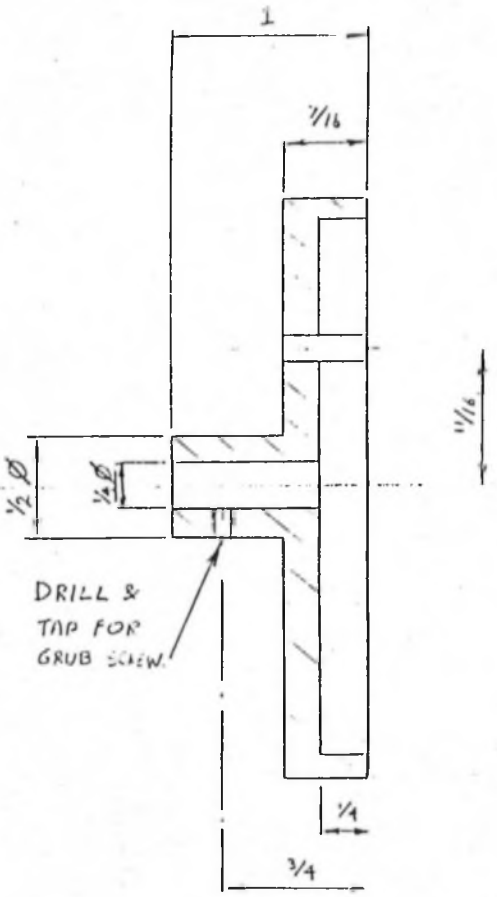

YELLOW
RED
GREEN
ORANGE
TRIG

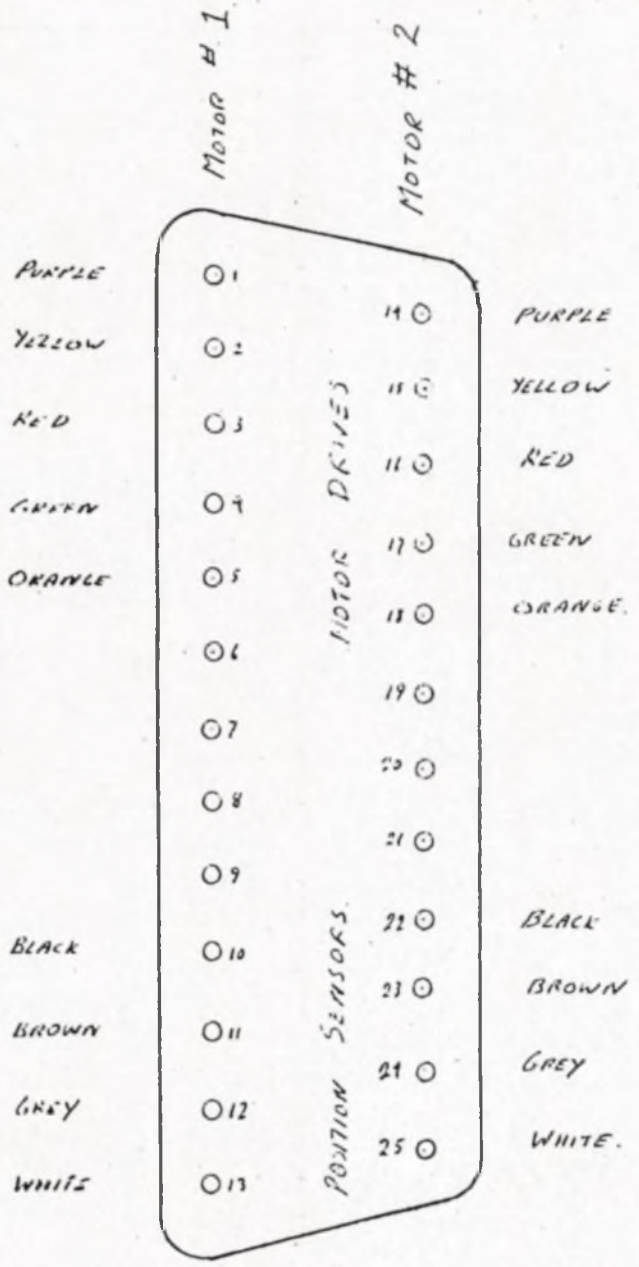
FIG 6 DRIVE COUPLING CUPS



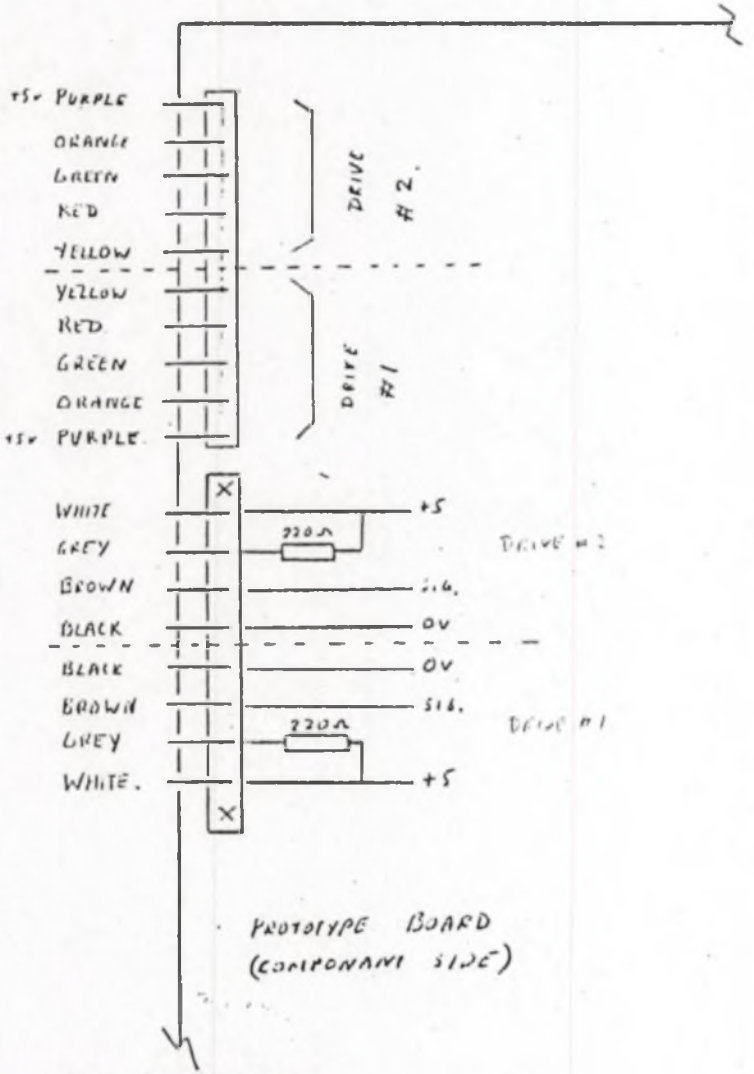


ALL DIMENSIONS IN INCHES

FIG 7 MOTOR CABLE CONNECTORS



25-WAY D-TYPE CONNECTOR.
(MOTOR FRAMEWORK END)



PCB CONNECTORS

the torque.

A framework was made up, as shown in figure 3, using aluminium angle strip with bolted joints. The motors used were manufactured by Superior Electric and have 200 steps per revolution.

Since both monochromators can be rotated through 360 degrees the driving motors need only rotate in one direction (as is the case with the internal drive). This has the advantage of eliminating any problems of backlash in the monochromator linkages and drive couplings. The motor cables were terminated on a 25 way D-type plug mounted on the framework.

The stepping motor driving circuits are based on the SAA 1027 motor driving IC and require few external components (see figure 4). The motor is stepped by the application of a trigger pulse (Figure 5). The rising edge of a this pulse initiates a 1.8 degree rotation of the output shaft in the direction defined by the level of pin 3, which in this application is always 0V, resulting in clockwise rotation at the non-drive end.

It was important that the motor framework was quickly detachable since the instrument was in regular use. However it was also most important that the drive motors were firmly mounted and that the drive couplings were positive and could not slip. After considering mounting the framework at various points on the instrument it was finally decided to use the arrangement shown in figure 6. This uses two driving cups directly mounted on the motor output shafts. These cups are machined out of aluminium so that they are a close fit over the knurled monochromator controls, thus locating the two motors at the same center spacing as the monochromators themselves. The motor torque is transmitted by means of a single 1/8" driving pin in each cup, which locates into a corresponding 1/8" hole drilled in the top of each monochromator knob. The weight of the motor / framework assembly is quite sufficient to hold the assembly in place on its four rubber feet which stand on top of the enamelled top surface of the instrument. Using this method the only permanent modifications to the instrument were the two 1/8" locating holes in the top surface of the monochromator knobs. The motor cables were connected to a 25 way D-type socket bolted to the framework (see fig 7 for details of pin allocations).

Having obtained a means of transmitting torque to the monochromators under computer control, it was then required to provide some means of calibration so that the setting of the monochromators can be determined at any time. Since the motor drives use only a single pin the relative positions of the motor shafts and the monochromators is unique, and cannot change even if the frame is removed and replaced, thus the rotation of the motor shaft can be found rather than that of the monochromator itself. This gives the advantage of being able to mount any measuring device on the motor framework assembly rather than on the instrument. The principal advantage of using stepping motors is that the degree of rotation from a starting point can be easily determined simply by counting the number of steps between the two points. Thus provided the rotation starts from a known datum point the exact position can be evaluated at any time. This

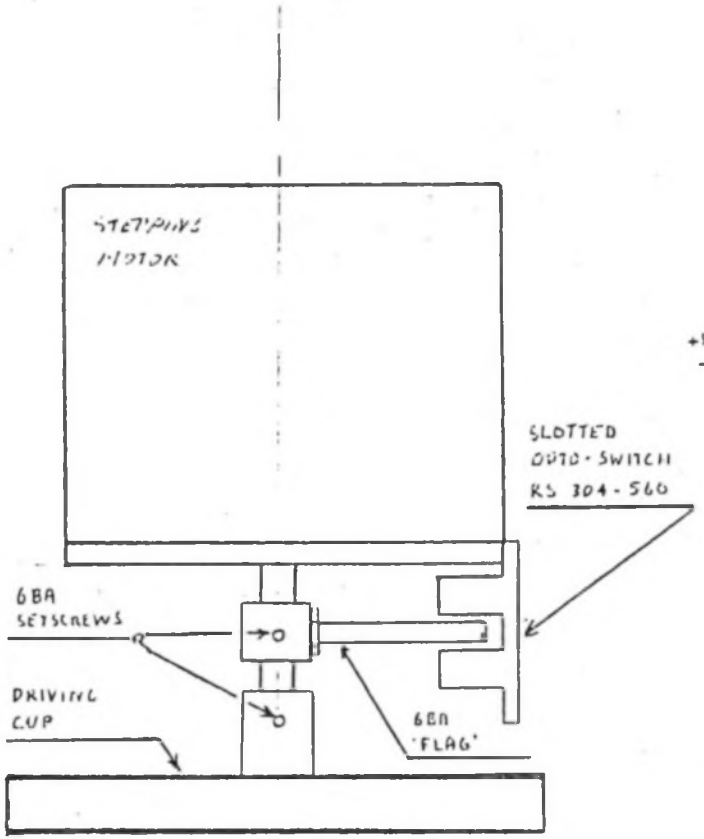
is very useful since it is far simpler to locate a fixed point than it is to actually measure an amount of rotation.

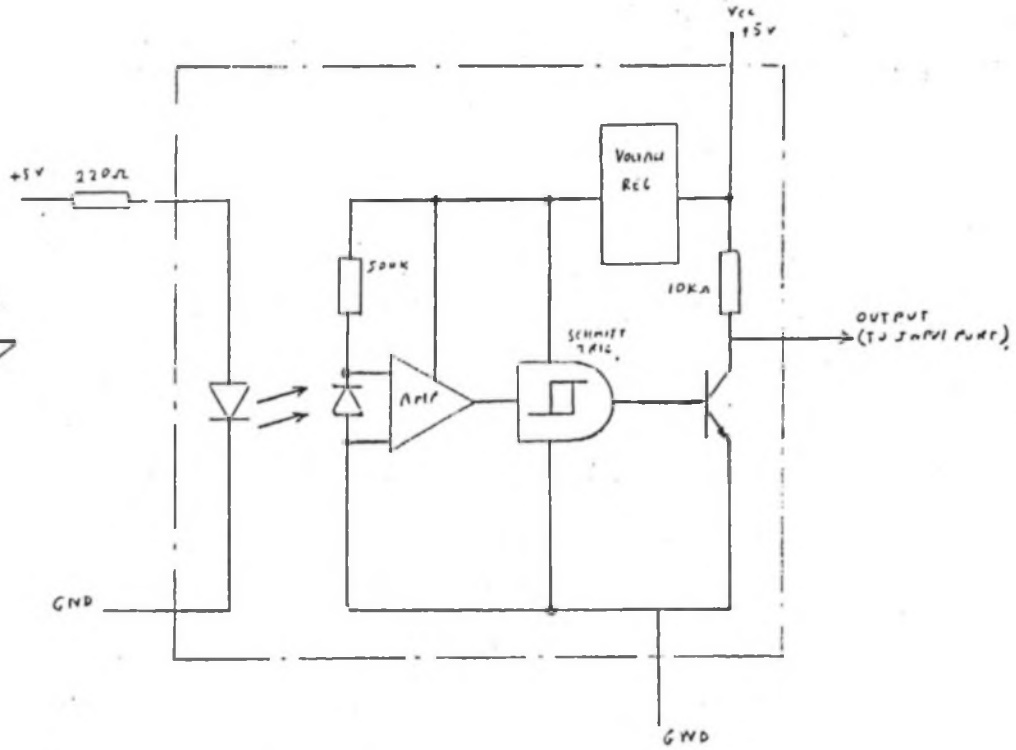
The method used to locate the datum point was to mount a flag on the motor shaft and use it to operate a slotted opto-switch (see fig. 8). The flag can be set to operate the switch at any point in the rotation however the best method is to set the switch operation to coincide with the 220nM position on the monochromator thus subsequent settings can be found by a multiplication and an addition, a task well suited to the computer. A problem which was not noticed until well into the project, was that the two drives had slightly different factors for wavelength per degree of rotation. Therefore, two different contants were needed to determine the wavelengths corresponding to particular rotations of the drives. Also, it meant that more readings would be taken in one dimension of the data matrix than the other, so that the data storage was made more complicated than if the drives had been symmetrical.

3.3 I/O PORTS

Though the cpu board carries an 8155 static read / write memory with I/O ports and timer, in order to increase the I/O capability and simplify the interconnection of circuit boards, a separate 8255 programmable peripheral interface was mounted on the development board. The 8255 gives up to three additional 8 bit I/O ports which is sufficient to drive all the required peripherals. It is quite simple to use and considerably simplifies the interfacing between the microprocessor and its associated circuitry. The 8 data lines are three-state terminals and can therefore be connected direct onto the data bus. It requires 4 consecutive I/O locations thus the two lower address lines are taken to the device itself. The remaining 6 address lines (8 I/O address lines in total) together with the M/IO are decoded, using TTL logic, so as to position the device in the four lowest locations in the I/O map (see figure 10). The only remaining inputs are the RD and WR which are themselves outputs from the 8085 cpu. However since the Quarndon Q-BUS is a general purpose bus system it does not carry these signals thus they must be regenerated from the available signals (E and RD/WR) (as shown in figure 9). The details of the port allocations can be seen on the A/D convertor diagram, figure 12, and Table 1

FIG 8 MOTOR POSITION SENSORS.





RS 304 560

SLOTTED OPTO SWITCH WITH LOGIC.

FIG 9 REGENERATION OF 8085 $\overline{RD}$ & $\overline{WR}$ SIGNALS FROM THE Q-BUS

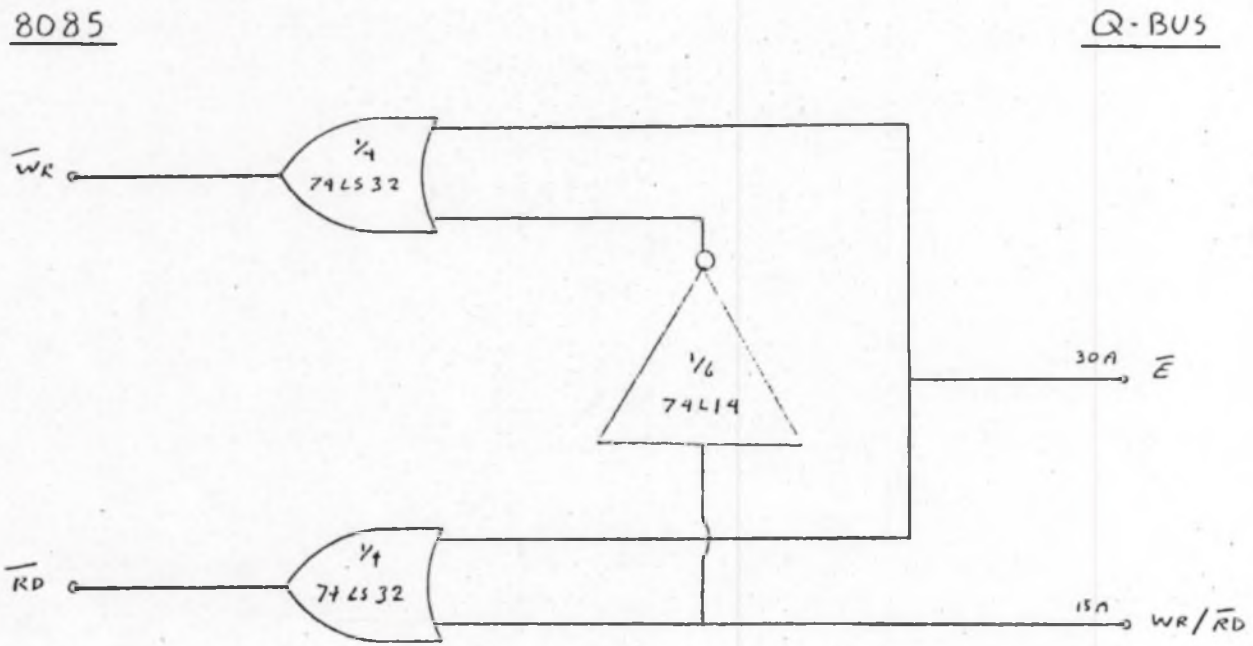


FIG 10. 8255 DECODER LOGIC

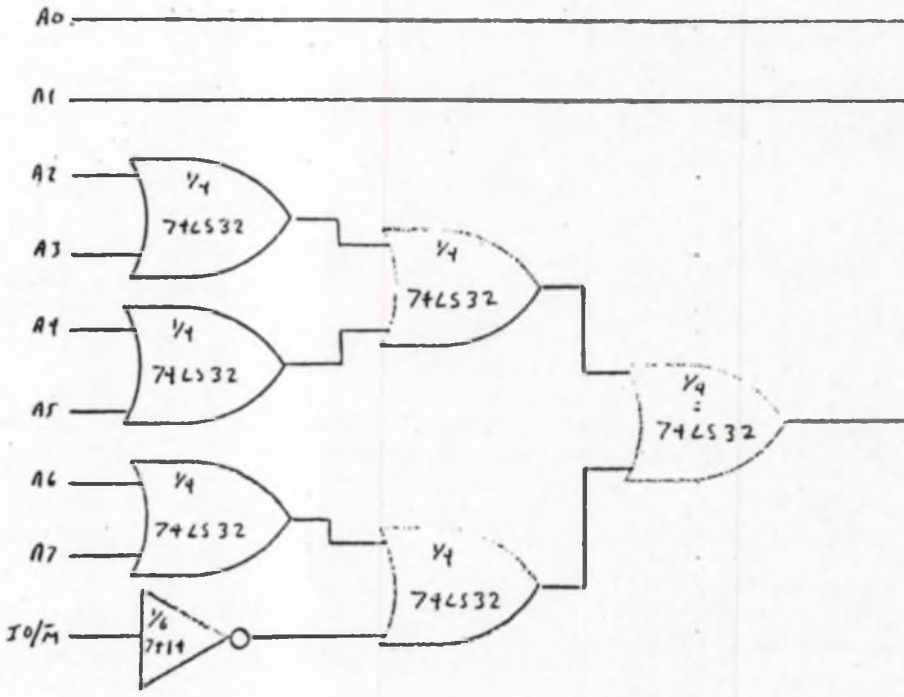
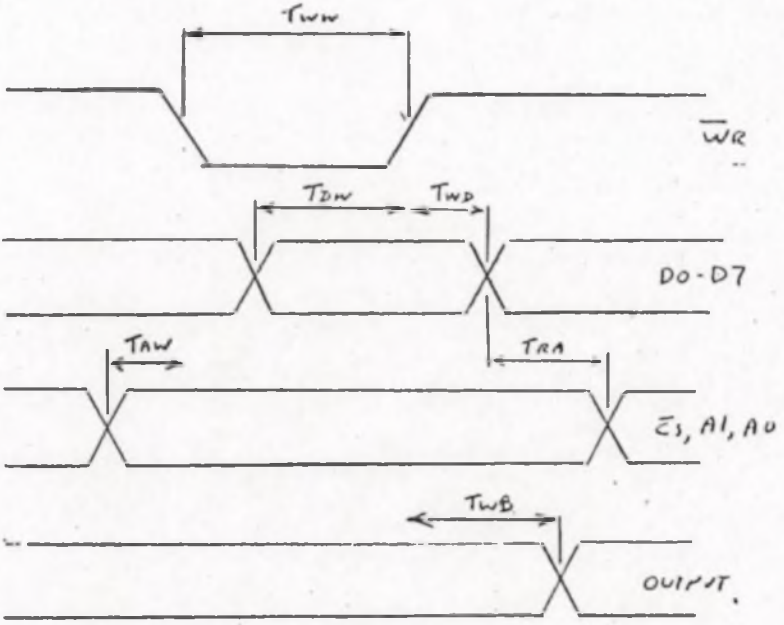
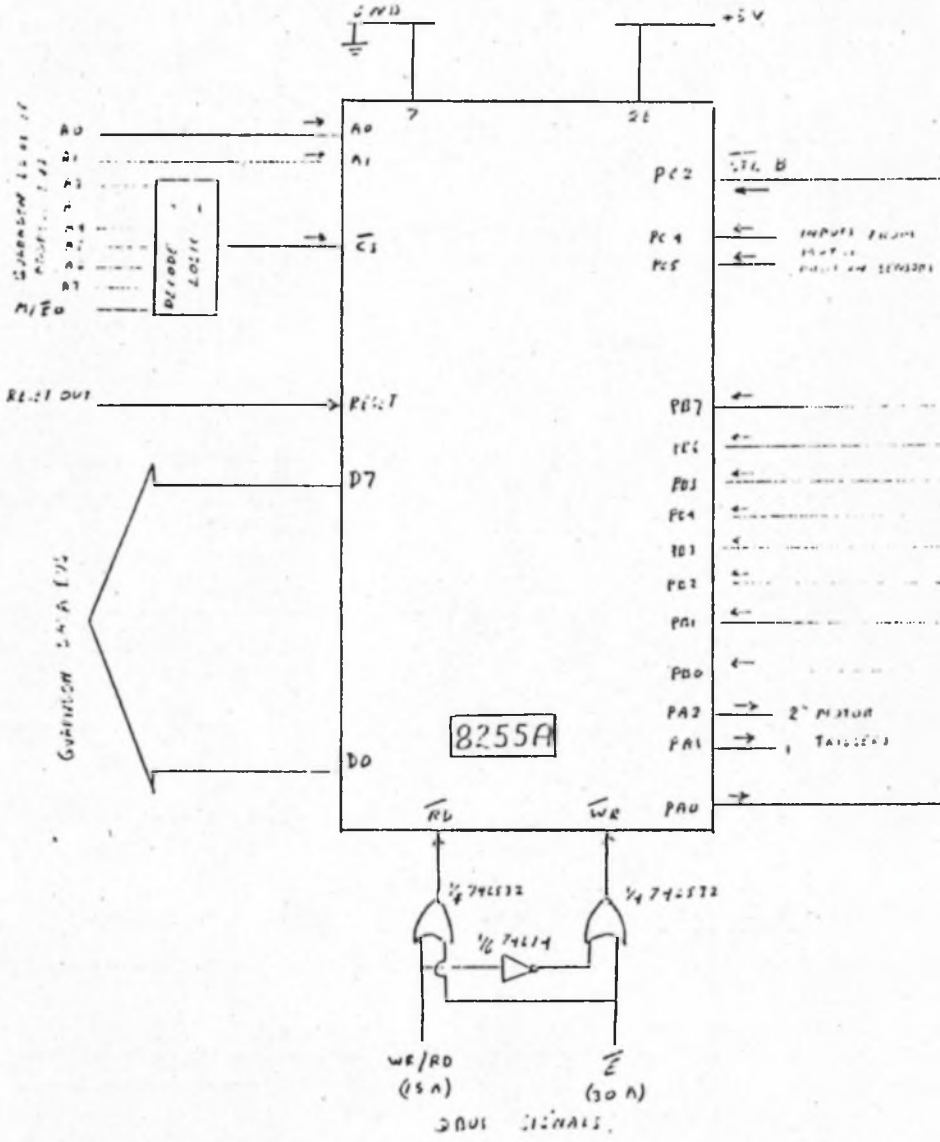


FIG 11. 8255 MODE 0 BASIC INPUT TIMING



TWW	WRITE PULSE WIDTH	> 300 nS
TDW	DATA VALID TO WRITE	> 100 nS
TWD	DATA VALID AFTER WRITE	> 30 nS
TAW	ADDRESS STABLE BEFORE WRITE	> 0 nS
TWA	ADDRESS STABLE AFTER WRITE	> 20 nS
TWB	WR=1 TO OUTPUT	< 350 nS

FIG 12 A/D CONVERTOR & I/O PORTS



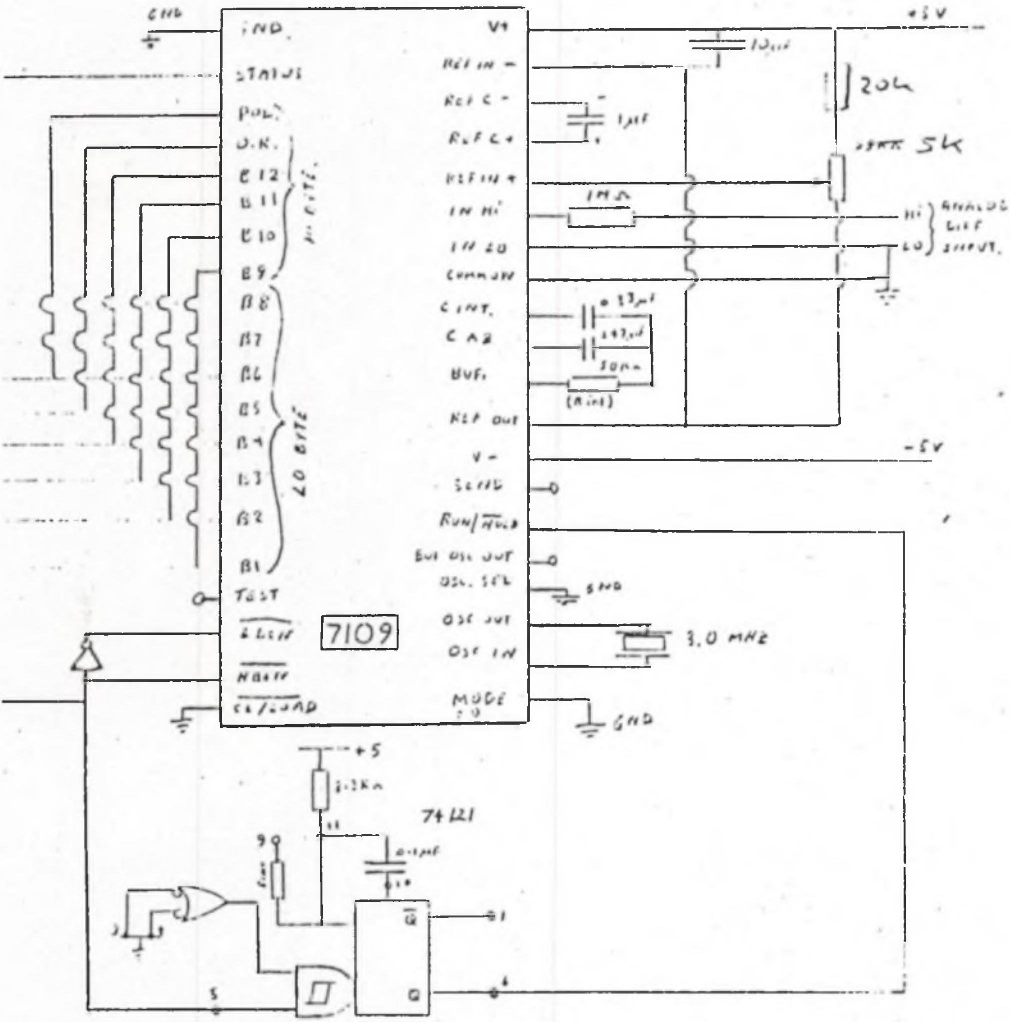

7109
GND
+5V
-5V
V+
IND
STATUS
POL
O.R.
B12
B11
B10
B9
B8
B7
B6
B5
B4
B3
B2
B1
HI BITE
LO BITE
TEST
REF IN -
REF C -
REF C +
REF IN +
IN HI
IN LO
COMMON
C INT.
C AZ
BUF.
REF OUT
V -
RUN/HOLD
OSC. SEL
OSC OUT
OSC IN
MODE
20k
5k
1MΩ
1μF
(R int)
HI
LO
3.0 MHz
+5
74121

TABLE 1 I/O PORT ALLOCATIONS

ADDRESS (HEX)	BIT	CHIP	FUNCTION
FA		8155	PORT B Data from Apple (Mode 4)
F9		8155	PORT A Data to Apple (Mode 4)
F8		8155	STATUS & CONTROL
0F		DL-1414	Display L2, CH1
0E			L2, CH2
0D			L2, CH3
0C			L2, CH4
0B		DL-1414	Display L1, CH1
0A			L1, CH2
09			L1, CH3
08			L1, CH4
07		DL-1414	Display L3, CH1
06			L3, CH2
05			L3, CH3
04			L3, CH4
03		8255	STATUS & CONTROL
02		8255	PORT C
	0		INTE B (Interrupt controlled by PC2)
	1		IBF B (Input Buffer Full)
	2		7109 STATUS TO STROBE B
	4		EM DATUM FLAG INPUT
	5		EX DATUM FLAG INPUT
01		8255	PORT B (MODE 1)
	0		7109 DATA B1 & B9
	1		B2 & B10
	2		B3 & B11
	3		B4 & B12
	4		B5 & O.R.
	5		B6 & POL.
	6		B7
	7		B8
00		8255	PORT A (MODE 0)
	0		7109 RUN / LBEN IF1, HOLD / HBEN IF 0
	1		TRIGGER TO EM MOTOR
	2		TRIGGER TO EX MOTOR

3.4 A/D CONVERTER

In order to manipulate and store the acquired data within the computer the analogue signal from the instrument must first be converted into a digital form. The basic requirements of the

A/D converter in this application are as follows:

1. 12-bit resolution to cover 1 part in 4096 dynamic range.

2. Good noise rejection - principally avoiding mains induced voltages.

3. Circuit simplicity.

4. Low cost.

5. Low speed, as the system speed is limited by the time constant of the photomultiplier amplifier.

6. Stability and temperature coefficient are not very important, as relative measurements are being made over a relatively short time scale.

The two principal types of A/D converter are the successive approximation type and the integrating type. The main advantage of the successive approximation type is its short conversion time thereby enabling fast sampling rates. However they may require several critical circuit components, sometimes including sample and hold circuits, and are usually quite expensive for 12-bit resolution, and are more sensitive to noise. The very fast conversion rate of this type of converter was not required in this application since the sampling rate would be restricted by the internal amplifier response anyway. The integrating type of converter is usually capable of 3-100 conversions per second which is quite adequate for this application and in addition it has such advantages as high inherant accuracy, non critical components, low cost, and very good noise rejection. The converter chosen was the Intersil ICL 7109 which is a dual slope integrating type of converter having 12 data bits and polarity and over range bits.

Interfacing this converter with the 8 bit input port is made simple by virtue of the byte organized output of the device. The 14 outputs are arranged as two bytes, the low order byte (B1-B7), and the high order byte (B9-B12, POL, & OR.). The two bytes are enabled by separate 'byte enable' input lines and the outputs are three - state i.e. the outputs of a disabled byte assume a high impedance state. The inherant noise rejection qualities of this type of converter are enhanced by the selection of an oscillator frequency which is an integral multiple of the principal noise source. In this application the principal source of noise is the 50 HZ mains supply, thus a 3 MHZ crystal was chosen as the oscillator source. This frequency is internally divided by a factor of 58 in the derivation of the converter clock. The total integration time is then given by the following:

T = (2048 clock periods) * 58 / (3 000 000) = 40mS.

This gives an integration time of two complete mains periods.

As can be seen in fig 12 there are few components in the analogue section however there are some which must be selected with care:

1. Integrating resistor, Rint: this must be high enough to keep the buffer amplifier and integrator in their linear range, but low enough to prevent undue leakage requirements on the board. The guide to selection given in the 7109 specification sheet suggests the following relationship: Rint = (full scale voltage / 20 uA). Since the analogue output voltage is 0-1V, a value of 50K was chosen.

2. Similarly the integrating capacitor, Cint, is chosen by the following : Cint = (2048 clock periods * 20 uA) / voltage swing. Additionally, Cint must have a low dielectric absorbtion in order to prevent rollover errors. A 0.33 uF polypropylene component was used.

3. Auto-zero capacitor,Caz: A large auto zero capacitor gives good noise rejection however since it, in parallel with Cint, forms an R-C time constant which determines both the speed of overload recovery and the error after the auto-zero phase it cannot be increased without limits. The specification guide suggests that 2Cint > Caz > (Cint/2).

4. Reference capacitor, Cref: The suggested value is 1.0uF.

The 7109 has an on board reference voltage source, sufficiently stable to be used as the main reference source at room temperatures. Since the instrument is only ever likely to be operated under laboratory conditions no external voltage reference was required. For calibration purposes a 20K resistor and 5k potentiometer is used as a voltage divider on the reference voltage input terminals. This reference voltage is set to calibrate the converter such that Vin (max) = 2 * Vref. Thus in this application, to give a full scale output (4096) at an input voltage of 1V the differential input voltage (pins 36 , 39) must be set to 0.5V.

The connections between the A/D converter can be seen on figure 12. The 8 data lines from the A/D converter connect directly into port B which is configured as a mode 1 input port. PC2 is the input strobe terminal for port B in mode 1. Port A is an output port and the least significant bit is used to control the operation of the A/D converter. In order to initiate a conversion PA0 is output high. This has two effects:

1. It enables the low order output byte, and puts the high order byte into the floating mode.

2. It triggers a monostable which provides a positive pulse at the RUN/HOLD pin on the converter. This pulse is timed by the values of C and R on the monostable and is set to 200 uS so as to start the conversion. As soon as it has started the STATUS output (pin 2) goes high and stays high until the conversion is complete. As it goes low it causes the low byte to be strobed into the input buffer of the 8255 port B. The data must then be read from port B before PA0 is output low thereby enabling the high byte which can then be read from port B. Once the high byte has been read the converter is ready for the next conversion.

3.5 DISPLAYS

The basic requirement of thc display was to show the current values of the three variables: excitation wavelength; emission wavelength; and output level. The latter is useful in the initial setting of the instrument gain controls at peak output to give a full scale reading of less than 4096. It was also considered to be desirable that the displays should have an alphanumeric capability so that it may be used to output user prompts.

The devices used are Litronix DL1414 4 digit 17 segment intelligent alphanumeric displays with memory, decoders, and drivers in a single dual in line package. Since the devices are self contained few external components are required. Each device has 4 digits therefore only 3 devices are used. Each individual digit can be addressed by means of the two least significant address bits (which are taken to the device itself). The remaining 6 I/O address bits together with the MEM/IO line and the WR line are decoded to give a unique chip select line (CS) to each device. The details of the decode logic can be seen in figure 13. The displays are mounted on the front panel behind a circularly polarised filter and they are connected to the development board by means of a 20-way Cannon insulation displacement connector and ribbon cable. The arrangement of display device addresses is shown in figure 14.

3.6 PARALLEL INTERFACE TO APPLE

The most obvious way to communicate between the Apple and the Quarndon system would be to use the two serial interfaces. However, the serial interface on the Apple was used for the HP 7470A plotter, so it was decided to use the parallel ports. This would also produce faster data transmission.

The interface uses the 8155 chip on the QMS 85-1185 board and a 6821 chip on the SSM AIO II board in the Apple. The 6821 has two parallel ports each with two handshake lines. Port A is used for data input to the Apple from the Quarndon, and port B is used for data output to the Quarndon. Port B is fully buffered on connector J1, including CB2, the handshake output, so no problems are encountered using a 2 metre ribbon cable. All the I/O lines on the Quarndon 8155 ports are buffered. Port A is not buffered, but as it is only used as an input, this is no problem. However, the handshake output line (CA2) does need buffering; fortunately, a spare gate was available on the AIO board on chip U1, a 74LS32, so the track was broken at point A-B, and the gate wired in as shown in Figure 15. The interconnections are shown in Table 2.

FIG 13 DISPLAY DECODE LOGIC

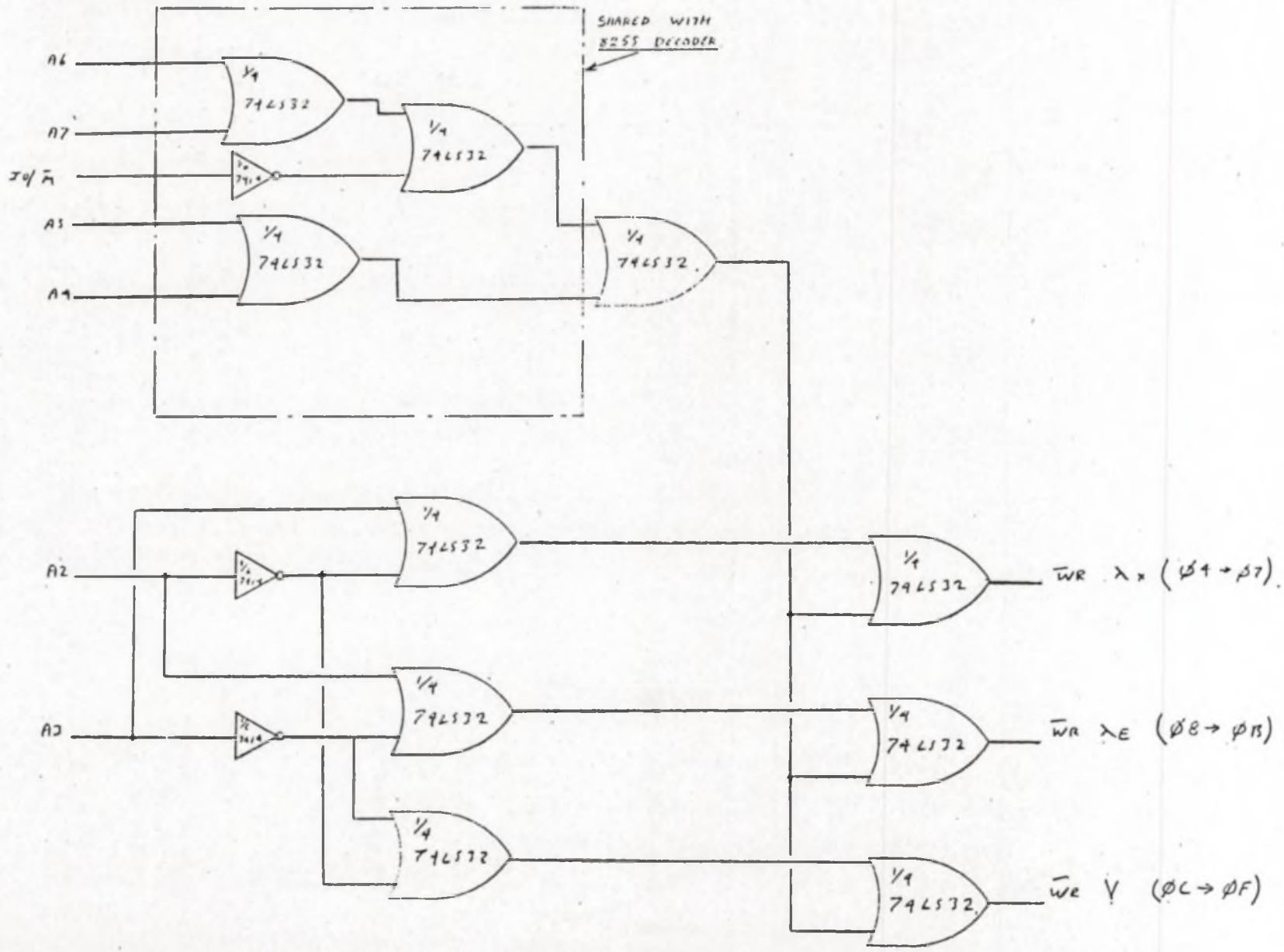


FIG 14 DETAILS OF DISPLAY DIGIT ADDRESSES.

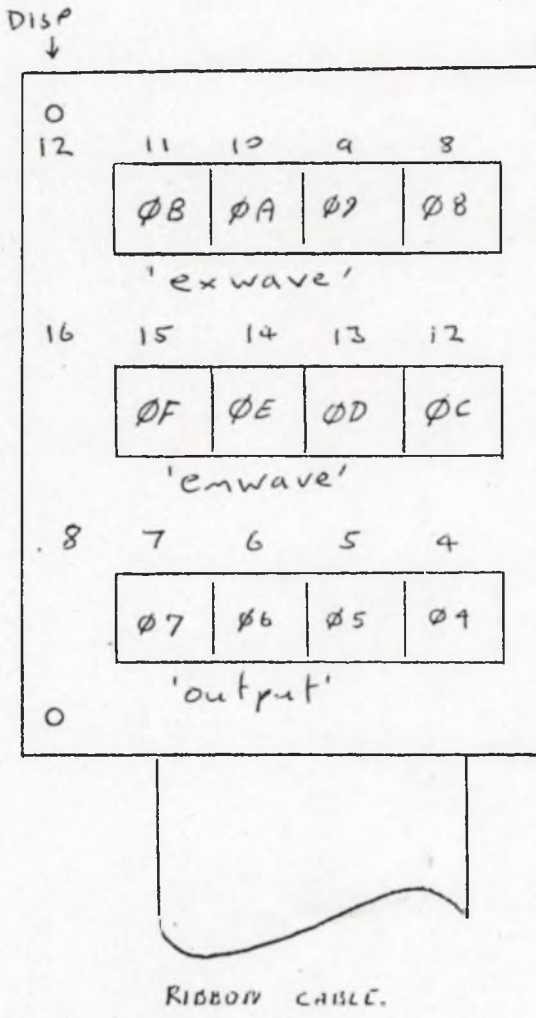


TABLE 2 Interconnections between Apple and Quarndon

APPLE 6821 PIN	CONN.	QUARNDON 3M CONN.	8155 PIN	FUNCTION
CA1	J2,12	19	<ABUF	Buffer full to Apple (See Note)
CA2>	J2,11	18	>/ASTR	Output Strobe to Quarndon
PA0	J2,25	15	<PA0	Data to Apple
PA1	J2,24	13	<PA1	"
PA2	J2,23	11	<PA2	"
PA3	J2,22	9	<PA3	"
PA4	J2,21	7	<PA4	"
PA5	J2,20	5	<PA5	"
PA6	J2,19	3	<PA6	"
PA7	J2,18	1	<PA7	"
CB1	J1,10	20	<BBUF	Buffer full to Apple
CB2>	J1,1	18	>/BSRT	Input Strobe to Quarndon
PB0>	J1,2	16	>PB0	Data to Quarndon
PB1>	J1,3	14	>PB1	"
PB2>	J1,4	12	>PB2	"
PB3>	J1,5	10	>PB3	"
PB4>	J1,6	8	>PB4	"
PB5>	J1,7	6	>PB5	"
PB6>	J1,8	4	>PB6	"
PB7>	J1,9	2	>PB7	"

> & < Means input or output buffered in direction indicated

Note: ABUF (Port A Buffer Full) has a momentary push button to ground, which is used to release the interface from lock-up for the case when the Apple misses the positive going edge of this signal which should tell it that data is available. This situation only occurs if the Apple has been switched off or reset during the time when the Quarndon is collecting data.

From the point of view of the Quarndon system, data is input using the two handshake lines as follows: when the Apple has put data into its output port, it pulses the BSTR line low. The Quarndon puts the BBUF line high, which tells the Apple not to change the data in its output port. The Quarndon then reads the data into a register, followed by sending the BBUF line low, so that the Apple can tell that the Quarndon is ready for more data.

Data output from the Quarndon is carried out in a similar way through port A. The Quarndon puts data into the output port, and sets ABUF high to inform the Apple that data is there to be read. The Apple then reads the data from its input port and pulses the ASTR line low. This causes the ABUF line to return low, and tells the Quarndon that data has been read and that more data can be written to its output port.

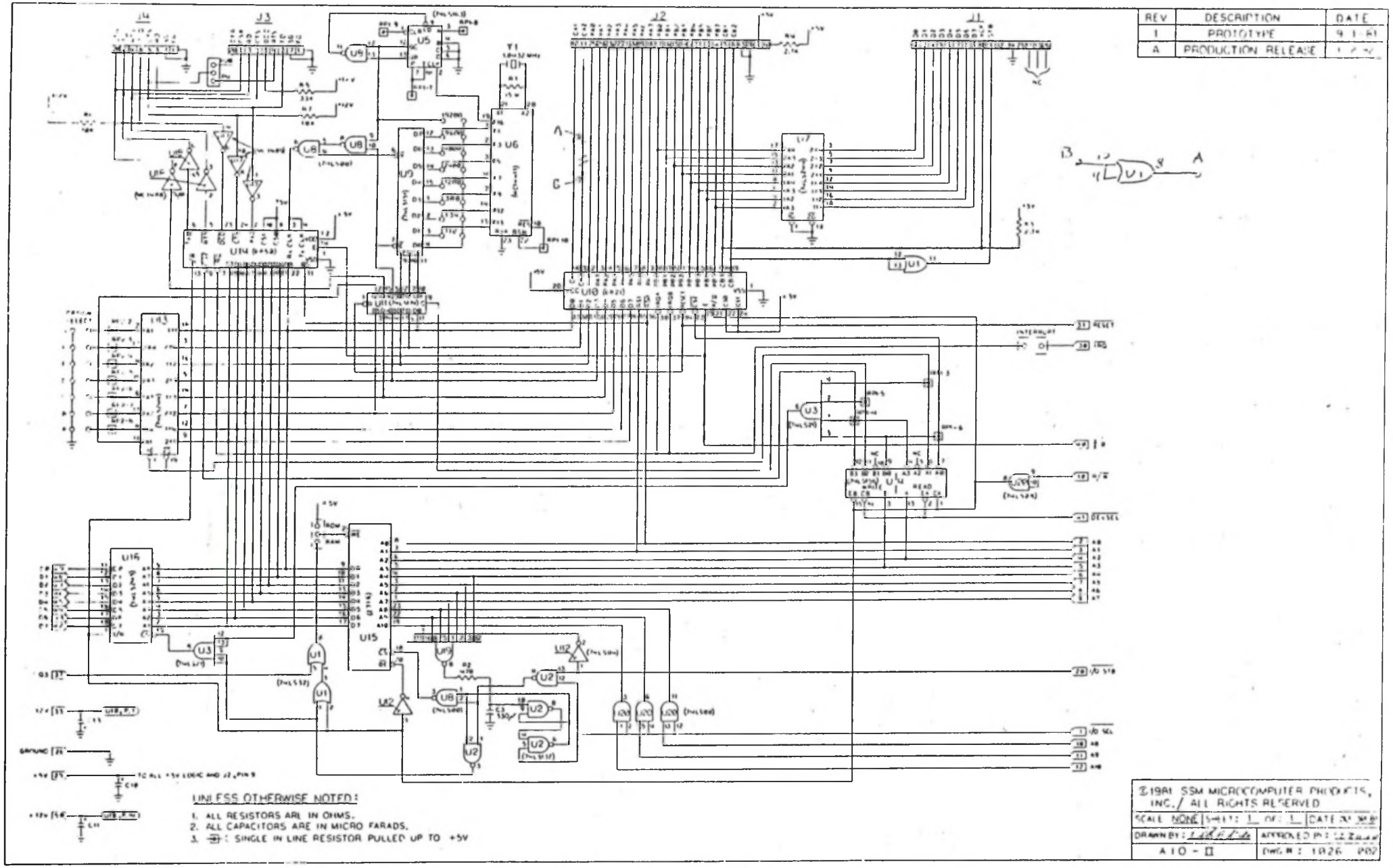


FIG 15. APPLE AIO II BOARD MODIFICATION

3.7 MODIFICATIONS TO FLUORIMETER

The major limitation on speed of operation of the automatic scanning system was the slow photomultiplier amplifier in the Perkin-Elmer fluorimeter. The best option for improving this would be to replace the amplifier with a more modern design. This could combine higher speed with lower noise. (Note: the amplifier uses a 12AU7 valve) The instument was speeded up by replacing the filter capacitors, C151, C152 and C153, with ones of 10 times lower value (e.g. C151 became 1nF, C152 became 10nF and C153 became 100nF), as shown in Figure 16. There are three capacitors to correspond to the three gain setting resistors for the different sensitivity ranges. The reduction in time constants resulting from these substitutions allowed the speed of scanning to be increased without smoothing of peaks. Noise as measured with an analogue chart recorder or oscilloscope was increased, but the integrating A/D effectively filters this. An active filter was tried, but gave little improvement.

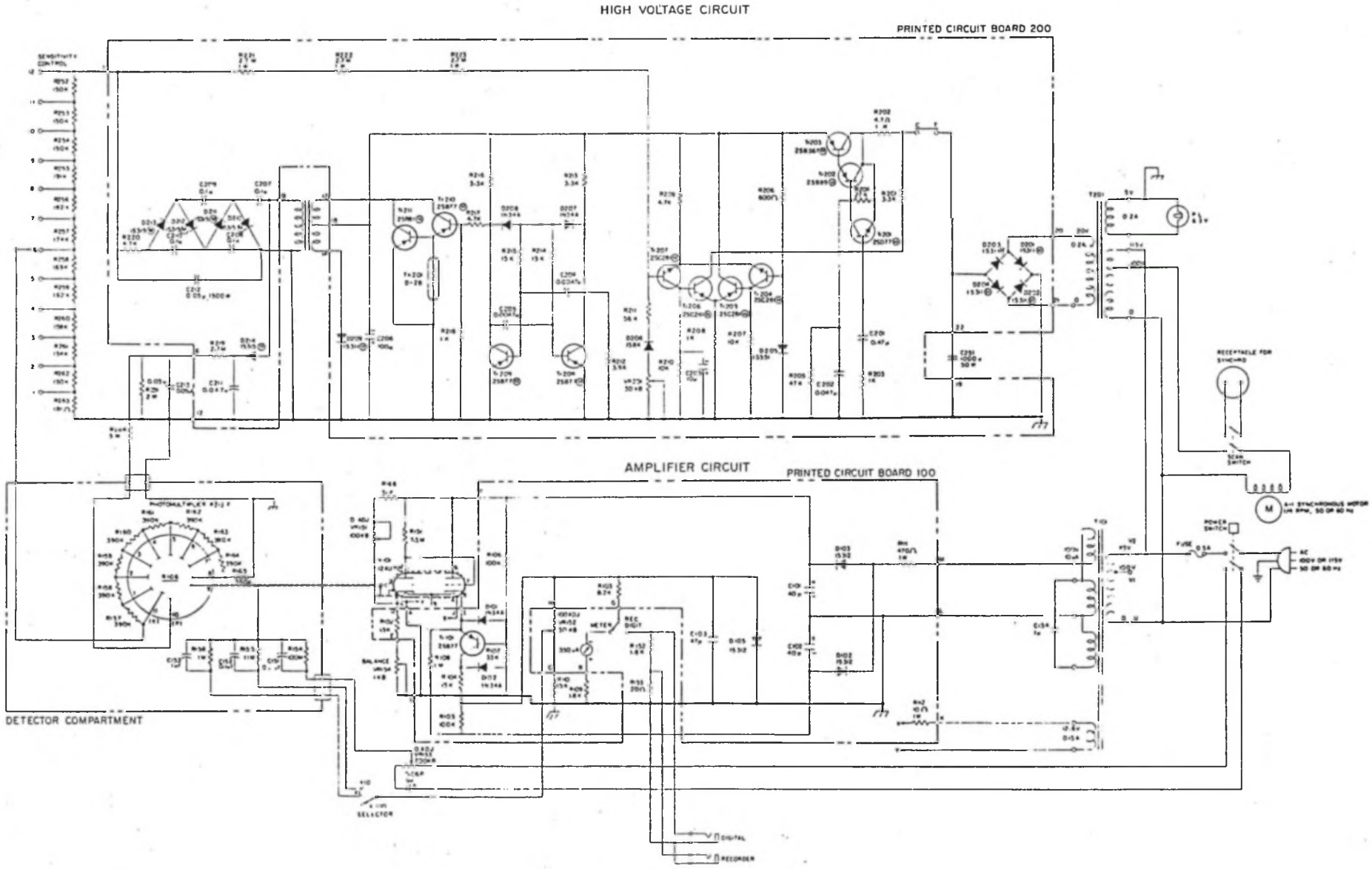


FIG. 16 FLUORIMETER MODIFICATION - Overall Electrical Schematic

4. SOFTWARE

Software for the system is divided into two parts: that which runs on the Quarndon system, which is written in Pascal MT+ and stored in EPROM; and that which runs on the Apple, which is written in UCSD Pascal (Apple Pascal 1.1) and 6502 assembler, stored on disk.

4.1 QUARNDON PROGRAM (QDRIVE)

4.1.1 DATA STRUCTURE

When the first versions of the software were written, data were collected as a symmetrical matrix of readings with a maximum size of 156 X 156. Two complications became apparent: firstly, the monochromator drives were not equal in nm per degree of rotation; secondly, if readings were taken over the whole range, those taken where the excitation wavelength was near the excitation wavelength would pick up very strong scattering peaks which would cause saturation of the amplifier.

To accommodate the different calibration factors a matrix of 156 X 150 was found to fit the two drive ratios between 220 and 780 nm.

The original 'square' matrix would generate 24,336 readings; each requiring 2 bytes (48,672 bytes in total). As only 50,175 bytes were available in RAM, the complete matrix could not be stored at one time, so the first half the data were read and transferred before the next block.

A method of solving these problems was devised, which relied on the fact that there were no useful data in the region where the excitation wavelength was greater than the emission. Referring to Figure 17 , the areas F and G were not needed. Therefore, instead of a matrix of 156 X 150 integers being set aside, one of 162 X 75 was used (24,300 bytes), called 'spectra'. The readings which would have been stored in area B are stored in F by using a 'fold-over' mapping function. Those which would have been in C are stored in E.

Other variables stored include:

speed, which determines delay between readings and hence the scanning speed;

no_steps, which determines the number of steps the motors move between readings (1 results in the most data, but corresponds to about 3nm which is considerably less than the bandwidth of the monochromators at 10nm);

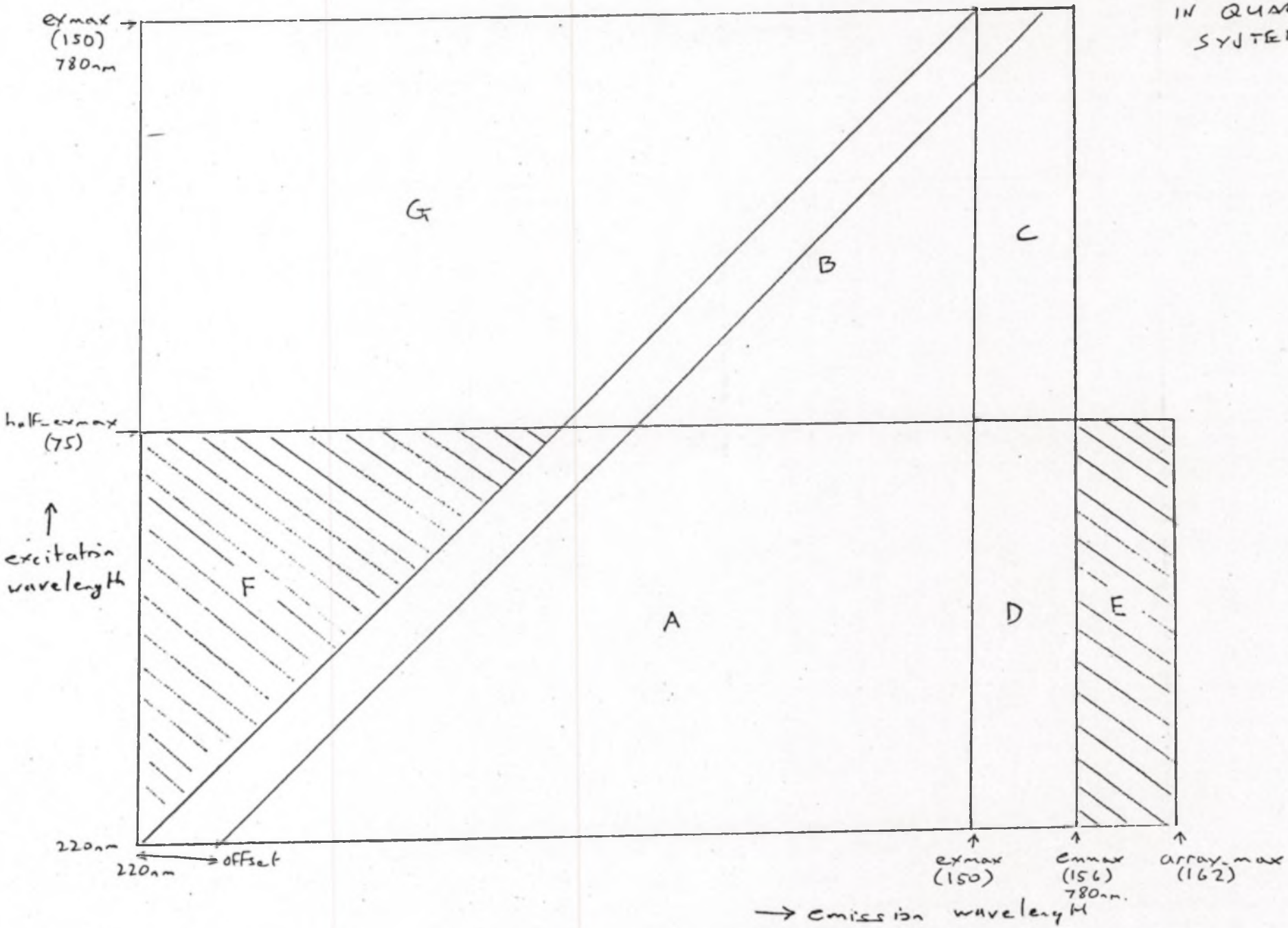
offset, which is the number of steps the minimum emission wavelength is offset from the excitation = emission line;

startex, which is the excitation wavelength to start scanning at;

stopex, which is the excitation wavelength to stop scanning at;

startem, which is the emission wavelength to start scanning at;

FIG. 17 DATA STRUCTURE IN QUANTUM SYSTEM



stopem, which is the emission wavelength to stop scanning at.

4.1.2 MAIN PROGRAM

The first line of the program (after the PROGRAM header) set the stack pointer, with {ZFEFF}. This puts the stack in the 8155 RAM, instead of the CP/M default.

The main part of the Pascal program starts by calling 'set_up' to initialise the I/O ports. A start-up message is then displayed on the LED displays ('FLUO SCAN V3.4'). The output from the fluorimeter is then measured and displayed. The drive flags are then moved so that they are clear of the opto-switches. The drives are then rotated until the flags are just breaking the light beams in the opto-switches. This procedure sets both drives to 220 nm and takes up any backlash in the mechanism.

The message 'WAIT FOR APPL' is displayed while the Quarndon system waits for a character to be received over the Apple/Quarndon parallel interface. Four options can be chosen by the Apple operator. If the Quarndon receives '*' then the communications to the Apple is checked by echoing a '*' back to the Apple (procedure 'check_comm'). If a 'V' is received the procedure 'movexem' is called to move the monochromators to specified wavelengths, and display a continuous reading of the emitted light. This is useful for setting the sensitivity controls on the fluorimeter.

The character 'S' calls procedure 'scan_and_dump', which carries out the scans of emission against excitation, followed by dumping the data back to the Apple. This process may take up to 1 hour, but the Apple may be used for other purposes during this time.

Reception of 'Q' brings the program to an end, and returns to the beginning of the main program.

4.1.3 PROCEDURE drive (no : INTEGER ; dr : drtype) ;

This moves one of the drives by 'no' steps. It checks if 'no' is between 0 and 200, and if so sends 'no' pulses to the appropriate stepping motor. The variable 'dr' is 'ex' for the excitation motor and 'em' for the emission motor.

4.1.4 PROCEDURE shift_flags ;

Moves the motors one step at a time until the flags are clear of the opto-switches.

4.1.5 PROCEDURE blank (disp : INTEGER) ;

Clears one line of the LED display. The line is chosen by 'disp', which is either 12 for the top line (exwavelen), 16 for the middle one (emwavelen), or 8 for the bottom one (output). The global variable 'location' is used to pass the address of the

actual character to be cleared to procedure 'wir'. (Note that this is bad practice, but is a legacy from the first test programs, and was not thought to be worth tidying up.)

4.1.6 PROCEDURE wir (ch : CHAR) ;

This writes a character to the LED display at address 'location-1', unless it is CR or LF.

4.1.7 FUNCTION read_port : CHAR ;

The purpose of this function is to read a character from the Apple via the 8155 port B in Mode 4 (Strobed I/O). It includes the function 'data_ready', which tests bit 4 of the status and control byte of the 8155 (Port B Buffer Full), returning TRUE if the buffer contains a character received from the Apple. The 'read_port' function waits until 'data_ready' is TRUE, then reads an integer from port B, converts it to a character and returns its value.

4.1.8 PROCEDURE write_port (ch : CHAR) ;

This sends a character to the Apple via the 8155 port A in Mode 4. It includes the function 'port_busy', which tests bit 1 of the status and control byte of the 8155 (Port A Buffer Full), returning TRUE if the port is not ready to accept a character from the Quarndon processor. The 'write_port' function waits until 'port_busy' is FALSE, then converts the character 'ch' to an integer, and writes the lower order byte to port A.

4.1.9 PROCEDURE writeno (no,disp : INTEGER) ;

This writes an integer to one of the three display lines chosen by 'disp'. It first clears the line, because characters remaining from the last display will not be cleared by short integers. The number is written using redirected output to the procedure 'wir'.

4.1.10 PROCEDURE writest (str:four_string, disp: INTEGER);

Uses redirected output to write a four-character string to one of the display lines.

4.1.11 PROCEDURE write_message (exmess,emmess,out : four_string);

This writes three four-character strings to the three displays; it is used to display prompts and messages.

4.1.12 PROCEDURE delay ;

Generates a short delay, usually used to allow a message to be read.

4.1.13 PROCEDURE set_up ;

Firstly, this procedure sets up the 8255 status/control word, by writing 10001111 to it. Bit 7 Hi makes the control word determine the I/O modes. Bits 6 and 5 Lo make port A Mode 0 (basic bit mode I/O). Bit 4 Lo makes port A an output port. Bit 3

set Hi makes the upper 4 bits of port C inputs. Bit 2 Hi makes port B Mode 1 (Strobed I/O). Bit 1 Hi makes port B input. Bit 1 has no effect on the lower 4 bits of port C, as these are defined for strobed I/O in Mode 1.

The next output instruction is OUT (sec_cont) := $05. This sets bit 2 of port C Hi. This line is used as the Status line from the A/D converter, to strobe data into port B. I do not see why it should be set during 'set_up' !

The next instruction writes 00001001 to the status/control word of the 8155 chip. Bits 7 and 6 Lo ensure no operation is done to the timer (which is not used). Bits 5 and 4 Lo disables the two interrupts (as the status word is polled to check for data available or port busy). Bits 3 and 2 set to 10 defines the mode to ALT 4 (both ports A and B in strobed I/O mode). Bit 1 Lo sets port B as an input, and bit 0 Hi sets port A as an output.

4.1.12 FUNCTION analog : INTEGER ;

Returns an integer corresponding to the analogue input to the system from the fluorimeter. It begins by setting bit 0 of port A Hi. This is the RUN/LBEN line to the 7109 A/D converter, which when pulsed Hi starts the conversion process. It remains Hi to enable the least significant byte of the A/D output.

The program must now wait for the STATUS line from the A/D to go Lo, indicating that conversion is complete. Firstly, the INTR B bit of the status register must be reset by a dummy read from port B, after the IBF B bit has been checked to see if data is ready in the port register. It tem waits until bit 1 of the status byte is Hi (IBF B), and hence the STATUS (or /STB) line has gone Lo; the byte in port B input register is then read to the 'lobyte' variable. Bit 0 of port A is then reset Lo, to enable the most significant byte of the A/D converter, and this value read into 'hibyte'. Bit 4 of this is checked for over-range, and if Hi 'OVLD' is displayed. Note that the sign bit is ignored. The four most significant bits are masked off, and the four least significant shifted into the upper byte of the integer (SWAP). The value 'analog' is then given by addition of lobyte and hibyte.

4.1.13 PROCEDURE zero (dr : drtype);

Sends the drive motor corresponding to 'dr' (ex or em) to the datum point at 220nm. It does this by stepping the motor until the flag bit is Lo. The start wavelength (220) is displayed on the appropriate line of the LED display.

4.1.14 PROCEDURE complete ;

Displays the messages 'END OF RUN', and 'RESTART SCAN'.

4.1.15 FUNCTION read_num : INTEGER ;

Returns an integer read from the Apple via port B. It reads one character at a time until it gets a CR or 4 digits have been

read. Any non-numeric characters are ignored.

4.1.16 PROCEDURE check_comm ;

Sends a '*' back to the Apple and displays 'COMM O.K.' on the LEDs.

4.1.17 PROCEDURE wri-steps ;

This is used to send information on scan speed, start and stop wavelengths etc. to the Apple. It starts by writing '?' to the Apple, and displaying 'STRT APPL READ'. It then waits until a '?' is received back from the Apple to confirm that the program is running which expects to receive this information. The data is then sent using redirected output to address 'app', previously set up as ADDR (writeport), so that the WRITELN procedure sends characters to the output port.

4.1.18 PROCEDURE settle ;

Generates a delay to allow for the amplifier to settle. The delay time is determined by the value 'speed', which was fed in by the Apple operator.

4.1.19 FUNCTION step_to_wave (pnt: INTEGER ;dr:dr_type): wave ;

This is used to convert a number of motor steps given by pnt to the corresponding wavelength. The drive type (ex or em) is also specified, as the calibration factors are different, as mentioned previously. The value returned is of type 'wave', which limits the range to start_wave (220) to stop_wave (780). A check is therefore made to the value returned so that rounding errors do not cause an out-of-range error.

4.1.20 FUNCTION wave_to_step(wave_length:wave;dr:drtype): INTEGER

This is the inverse of the previous function and returns the number of steps corresponding to a given wavelength. It rounds up to the next step if there is a fractional part to the result. This means using the MOD function, which causes a problem in a system which does not have CP/M mounted, as in this one. The standard MT+ 'MOD' function calls a CP/M system function if there is a divide by zero error. We have written a modified function which calls a new @ERR error procedure to display an error message on the LED display, and continue; not reset as the standard @ERR procedure does (See section 4.2)

4.1.21 PROCEDURE scan_and_dump ;

This is the main scanning and data transfer procedure. The main block of this procedure (starting at BEGIN {scan_and dump}) starts by sending the character 'S' back to the Apple to acknowledge that the scan process has begun. It then reads the following from the Apple: number of steps between readings (no_steps); the scan speed (speed); the wavelength to start the excitation scan at (startex); the wavelength to stop the excitation scan at (stopex); the wavelength to start the emission scan at (startem); the wavelength to stop the emission scan at (stopem); and the offset from the excitaion = emission wavelength

line at which to start emission scanning (offset). The scanning is then carried out by calling the procedure scan, with the passed Boolean variable FALSE, so that no dumping of data occurs this time.

When the scan process is over, the 'wri_steps' procedure is called to send the start and stop, no_steps etc data back to the Apple (which may have been doing something else during the scan).

The procedure 'scan' is again called; this time with the Boolean TRUE, so that the data matrix is scanned but readings are not taken, nor are the motors moved, but the data are transferred to the Apple.

The wavelength drives are now returned to the datum positions, using 'zero'.

The next sections describe the procedures local to 'scan_and dump'.

4.1.21.1 FUNCTION spec_point (expoint,empoint:INTEGER):INTEGER;

As discussed in the section on data structure, the readings are stored in a folded over diagonal matrix. The purpose of this function is to make it appear that the data are stored as a normal rectangular matrix. The two co-ordinates expoint and empoint are passed into the function, and the reading returned, in a similar way to the way a value would be picked out of a normal matrix with: 'reading := spectra [expoint,empoint]'. The function maps the areas which are outside the limits of the matrix 'spectra' into unused areas of 'spectra'. Looking at Figure 17, any points in area B are read from area F, and any points in area C are read from E.

4.1.21.2PROCEDURE put_spec_point(expoint,empoint,number:INTEGER);

This is the mapping procedure which stores raedings into the folded over diagonal matrix. It serves a similar function to using: 'spectra [expoint,empoint] := reading' for a normal matrix. The mapping is exactly as in the previous function.

4.1.21.3 FUNCTION first_ex_point : INTEGER ;

Returns the step number corresponding to the starting point on the excitation scan. This is merely the conversion from wavelength to step, as there is no offset to calculate, unlike the emission scan.

4.1.21.4 FUNCTION last_ex_point : INTEGER ;

If the 'offset' is greater than emmax (156) - exmax (150), then the last excitation point will be less than exmax by the value of 'offset'.

4.1.21.5 FUNCTION first_em_point : INTEGER ;

Returns the starting point for the emission scan. This will increase as the value of excitation wavelenth increases, so that the emission wavelength is always less than the excitaion

wavelength by an amount determined by 'offset'.

4.1.21.6 PROCEDURE scan (dump: BOOLEAN) ;

This is a dual purpose procedure: it is used to collect data if 'dump' is FALSE; and to dump data to the Apple if 'dump' is TRUE.

If 'dump' is true, the procedure begins by calling 'start_dump', which writes 'DUMP OF DATA' on the LED display, sends 'A' to the Apple, and waits for an 'A' back. If 'dump' is false, then the excitation drive is zeroed to the datum point (220nm). The maximum count in the excitation direction (new_exmax) is found by calling 'last_ex_point', and the pointer to the matrix in the excitation direction (expoint) found by calling 'first_ex_point'. The equivalent of this in terms of wavelength (curex) is then calculated. If 'dump' is false, then the excitation drive is moved 'expoint' steps to put it at the start point.

A nested 'WHILE' loop structure is then entered. The outer loop scans in the excitation direction, and the inner in the emission direction. If 'dump' is false, at each point a value is read from the A/D converter and put into the matrix, followed by moving the emission drive by 'no_steps'. If 'dump' is true, the value is read from the matrix and written to the Apple using redirected output. At the end of each inner loop, and consequently each emission scan, the excitation drive is stepped by 'no_steps' if 'dump' is false, otherwise '8888' is written to the Apple to signify the end of an emission scan (Note that the maximum real reading from the A/D is 4095).

At each point, the wavelengths are calculated and displayed on the LEDs, as well as the value stored in the matrix at that point. At the end of the last emission scan, '9999' is sent to the Apple if 'dump' is true, to signify the end of the complete scan.

4.1.22 PROCEDURE movexem ;

This procedure is used to move both monochromator drives to specific wavelengths, under the control of the Apple.

It first acknowledges the Apple by sending a 'V' back. It then reads the next character from the Apple, which will be an 'X' if the excitation drive is to be moved, and an 'M' if the emission drive is to be moved. It reads the wavelength to be moved to from the Apple, and displays 'MOVE TO 300', or whatever is the chosen wavelength. It then calculates the number of steps to be moved (which will be different for the two drives), and moves the specified drive that many steps. This may involve returning to the datum point first (220nm) if the drive is already at a greater wavelength than that to which the drive must move. The two wavelengths at which the drives are now at are displayed.

The procedure then waits for a character from the Apple. If this is 'C', the system takes a reading from the A/D converter, displays it on the LEDs, and writes it to the Apple. This process

is repeated until the Quarndon receives an 'F' from the Apple. At this point it displays 'DRVS MOVED', and returns to the main program.

4.2 ERROR TRAPPING MODULE

As mentioned in 4.1.20, the standard error trapping procedure in Pascal MT+ does a direct call to CP/M. For this system which has the program stored in EPROM, and no CP/M BIOS (Basic Input Output System), it is necessary to provide an alternative @ERR procedure to link in preference to the standard one. This is contained in the module ERRMOD.

It uses the procedures 'delay' and 'writest', which are externally declared as they are compiled as part of QDRIVE.

4.2.1 PROCEDURE @ERR (error : BOOLEAN; errnum : INTEGER) ;

When this procedure is called with 'error' true, one of three error messages is displayed on the LEDs. Only three errors are possible, as there is no disk or file handling. These are:

Error 1 : message 'DIV BY ZERO';

Error 3 : message 'STR OVER FLOW';

Error 4 : message 'ARRY SUBR ERROR'.

The standard @ERR does a reset after an error, but this version continues the program flow after the error message and a delay, in the hope that the error is non-fatal. In fact, no errors occur in the de-bugged version of QDRIVE, but the procedure was tested by generating errors on purpose.

4.3 MODULE DIVMODQ

The MT+ @MOD procedure calls CP/M also, so this was modified to call the new @ERR procedure. The assembly code source for DIVMOD is supplied with MT+. It was simple to call @ERR instead of the direct CP/M function to write the error message. One wonders why Digital Research didn't do this!

4.4 LINKING THE PROGRAM MODULES

When the program has been successfully compiled, it must be linked with the Pascal library and the new error trapping modules. The Linker command string is :

```
QDRIVE28,ERRMOD,DIVMODQ,PASLIB/S/M/E/D:4000/P:100/H:100
```

Because ERRMOD and DIVMODQ are linked before the PASLIB, the new versions of @ERR, @DIV and @MOD are linked in preference to the ones in PASLIB. The data area starts at 4000H, and the program area at 100H (Note: the EPROM is programmed so that the first instruction is a jump to 100H (or fill the first 100 instuctions with NOP). The final version of QDRIVE (V3.4) used 201DH (8221 decimal) bytes of EPROM, and 6400H (25,600 decimal)

bytes of RAM.

4.5 APPLE PROGRAM (READFL)

The program on the Apple is written in Apple Pascal V1.1, which is a version of UCSD Pascal, and as such produces compiled pseudo-code (p-code) which is interpreted at run-time by a p-code interpreter.

4.5.1 DATA STRUCTURE

The most obvious way to store the spectra is as a large matrix, but this would take up a vast area of memory. Therefore, the structure used was to store each emission scan as a linear array (gridline : ARRAY [0..maxindex] OF INTEGER), and save the set of these on disk as a file of type 'gridfile', a FILE OF gridline.

For each file of type 'gridfile', called 'ourgridfile', a file 'ourlabel', of type 'labelfile', is stored, containing a record with the following data:

'title' and 'name': two strings to identify the scan and operator;

'today': today's date ('date' is a predefined data type);

'nosteps': the number of drive steps of 1.8^{o} between readings;

'startex,stopex,startem and stopem': start and stop points for the two wavelength drives;

'speed': parameter to set the delay between readings, and hence the scanning speed.

'offset': parameter to set the offset of the starting points for the emission scans from the excitation = emission line.

Another file of type 'labelfile' is kept ('lastlabel'). This keeps are record of the label information used by the last scan. It is used as defaults for the next scan, to save re-entry of identical values.

The source text for the program consists of three text files: READFL21.TEXT, RDFL21EXT.TEXT, and PLOT.TEXT. The latter is used for plotting a simple contour map of the data. It was written by M.A.Hurley and N.B.Hetherington, and will be described elsewhere.

4.5.2 MAIN PROGRAM

The main program begins by calling procedure 'portset', an external assembly code procedure which sets up the 6821 parallel port chip on the Apple AIO II. The 'labelname' and 'filename' strings are initialised to blank. A header message and instructions on what do with the fluorimeter are displayed, followed by the main menu:

S)tart scan
M)ove monochromators
R)ead data from Fluorimeter
P)lot data from File
G)et data from file and display
E)xit to Pascal while fluorimeter runs
C)heck communications
Q)uit

The operator chooses one of these options by hitting the appropriate key. The program repeats this process until either 'E' or 'Q' is hit. If 'Q' is chosen, the character 'Q' is written to the Quarndon system, to tell it to reset. To do this the Quarndon must have finished a scan and be waiting for a character from the Apple, or else the system will wait until it has finished the scan. If a scan is in progress, but you wish to exit to the Pascal operating system to carry to use other programs then press 'E', which exits without sending a character to the Quarndon.

The first option ('S') checks the communications with the Quarndon, to make sure it is on and the connection is sound. It then calls 'startscan', which does what it says. The second option ('M') calls 'movexem', to move the drives to specific wavelengths and display the emission intensity at that point.

The third option ('R') calls 'readdata', to read the data from the Quarndon system. 'P' is used to call 'plot' to do the contour plot. 'G' calls 'getdata' to get data from a file instead of direct from the fluorimeter. 'C' does the simple communications check.

4.5.2 PROCEDURE writeport (ch : CHAR) ;

UCSD has no low level extensions which allow you to manipulate memory locations and bits directly (unlike Pascal MT+), so short assembly code routines are used for I/O to the parallel port.

'Writeport' is an external assembly code procedure which sends a character 'ch' to the 6821 port B. It then pulses CB2 Lo to strobe data into the Quarndon 8155 port. This results in the Buffer full line going Hi, and remaining so until the 8155 buffer has been read, and is again ready for data. Therefore, writeport waits for CB1, which is connected to the Buffer Full line, to go Lo before returning to the calling procedure.

4.5.3 PROCEDURE portset ;

This is another external assembly language program, which is used to set up the I/O modes of the 6821 chip. Port A is set to all inputs, with CA2 a directly controlled output, and CA1 an input bit latched on a positive going edge. Port B is set to all outputs, with CB2 a directly controlled output, and CB1 an input latched on the negative edge.

4.5.4 FUNCTION readport : CHAR ;

This function returns a character read from the Quarndon parallel port, and carries out the hand-shake procedure. It first waits for bit 7 of the Status/control register to go Hi, signifying data has been written to the port, and that CA1 (A Buffer Full) has gone Hi. It then reads the register A, and acknowledges it to the Quarndon by pulsing CA2 (and hence /ASTR) Lo. The character is then pushed on to the stack, to return to the Pascal calling procedure.

4.5.5 PROCEDURE pause ;

This is a simple delay, usually used to allow the operator to read a message.

4.5.6 FUNCTION readnum : INTEGER ;

This is identical to 'read_num' in the QDRIVE program, and is used to read an integer of up to 4 digits from the Quarndon system, via the parallel port.

4.5.7 PROCEDURE writenum (num : INTEGER) ;

When we wanted to write numbers to the parallel port on th Quarndon system, we could use redirected output, with Pascal MT+. Apple Pascal does not have this facility, so 'writenum' was used. It sends up to four digits, terminated by CR and LF.

4.5.8 PROCEDURE getfilename ;

This is a procedure to prompt the user to provide a file name for numeric data storage, and storage of the 'label' information. It uses IORESULT to error check, ensuring that the user specifies a valid name. Data are stored in two files: for example FLUOR:ASTER.DATA and FLUOR:ASTER.L.DATA (the latter being for the 'label'). Note that it makes use of the procedure 'directory', which is added to the library, and displays the disk directory.

4.5.9 PROCEDURE startscan ;

The main body of this procedure is used to send information on the limits of the scan, speed and offset to the Quarndon; the scan is then started. The procedure starts by seeing if there is a file on disk containing the label information from the last scan. If so, it will use the values from this as defaults. Most of them are likely to be the same (e.g. operators name, date, scan speed), so this will speed up the entry of the next set of scan parameters. If an I/O error occurs when an attempt to read the 'lastlabel' file, it is assumed that this is the first time the program has been used, and no 'lastlabel' exists. In this case, the label data are initialised to default values.

The procedure 'input_parameters' is then called to allow the user to change any parameters he/she wishes. When this is complete, the new paramters are written to the 'lastlabel' file (called £4:LASTLAB.DATA).

The user is then prompted to start the scan by hitting the space-bar (presumably after setting up the sample in the fluorimeter). An 'S' is sent to the Quarndon system, followed by the scan parameters. After the message 'Scan in progress', the procedure returns to the main menu.

4.5.9.1 PROCEDURE input_parameters ;

Firstly, procedure 'get_steps' is called to prompt the user to key in the number of steps between readings. This procedure ensures that 'nosteps' is between 1 and 9, and tells the user what this means in terms of wavelength for each drive.

Procedure 'get_offset' is then called to get the value of 'offset' to determine the offset from the excitation = emission line. The scan speed is then entered, followed by calls to 'get_st', which gets the start and stop wavelengths. These are checked to ensure that the stops are greater than the starts, and re-entered if not.

The procedure then returns to 'startscan'.

4.5.10 PROCEDURE readdata ;

The purpose of this procedure is to read data from the Quarndon system. The main body begins by calling 'getfilename'. It then updates the label information by reading 'lastlabel' from disk (if it is there). 'Input_label' is then called to check and update, if necessary, the label information (date, operator etc.). The procedure waits for a '?' from the Quarndon, and then the rest of the label information is read from the Quarndon (speed, offset, starts and stops etc.). This information is written to both the 'ourlabel' and 'lastlabel' files.

The procedure 'read_block' is then called to read the block of emission values from the Quarndon. The procedure then returns to the main menu.

4.5.10.1 PROCEDURE read_block ;

This procedure is used to read a block of data from th e Quarndon system. It starts by sending an 'A' to the port, and waits for an 'A' back. It then goes through the array of 'ourgridfile^', putting each value to zero. Values are then read into the array until a value of '8888' is received to signify the end of the first emission scan. The array is then written to the data file 'ourgridfile'. This is repeated until a value of '9999' is received to signify the end of the last emission scan, at which point the file is close and locked.

4.5.11 PROCEDURE movexem ;

This procedure is used to move either the excitation drive or the emission drive to a specific wavelength.

It begins by writing a 'V' to the Quarndon, and waiting for another 'V' to be returned as an acknowledgement. It then asks the user which monochromator he/she wishes to move, and to what

wavelength. Either 'X' for excitation or 'M' for emission is written to the Quarndon, followed by the wavelength the user has input. This causes the appropriate monochromator to move to the specified wavelength.

The procedure then repeats the following steps until the user presses a key: a 'C' is written to the Quarndon to tell it to do a conversion; the A/D reading is read from the parallel port using 'readnum'; and the reading displayed (it is also displayed on the Quarndon LED display). When a key is pressed, the Apple writes 'F' to the Quarndon, instructing it to finish converting; the procedure then returns to the main menu.

4.5.12 PROCEDURE check_comm ;

This procedure tells the user to check that the Quarndon system is on and at the start of its program (eg switch on or reset it). The Apple then sends a '*', and waits for a '*' back.

4.5.13 PROCEDURE getdata ; (on RDFL21EXT.TXT)

The function of this procedure is to get data from a file and display and print these data.

The main body of this procedure begins by calling 'getfilename' to prompt the user for a file name (the last one used is taken as a default). The label information from this file is then displayed on the screen by calling 'writelabel' with 'CONSOLE:' as the filename for output passed to 'writelabel'. This displays the title, operator, date and all the scan parameters. The user is asked if this is the required data set, and if so, whether he/she wishes to display it on the screen, and also if he/she wishes to print it. If the user says this is not the set of data he/she wants, he/she is asked for another filename by calling 'getfilename' (Note that 'getfilename' includes a directory procedure, so the user is not looking for file names in the dark!).

If the user specifies that data should be printed as well as displayed, the Boolean variable 'print' is set true, and 'writelabel' again called, this time with 'PRINTER:' as the file name passed in for output.

The numeric data consisting of the file of emission scans ('ourgridfile') is then displayed. For each array of emission readings, it is necessary to find the first actual reading, because of the way data are collected as a diagonal matrix, with no readings where the excitation wavelength is greater than the emission wavelength. Account must also be made of the offset and specified start wavelengths for both emission and excitation scans. The functions 'first_em_step' and 'first_ex_step' are used to do this in a similar way to their equivalents in the QDRIVE program in the Quarndon.

The values read from the arrays are displayed and printed in 8 number lines, using 'count' as a counter. The wavelengths are also computed from the array indices using 'step_to_wave'.

The display (and print) process can be stopped at the end of

any emission scan by pressing any key, as ´KEYPRESSED´ is checked before each scan array is read from the file.

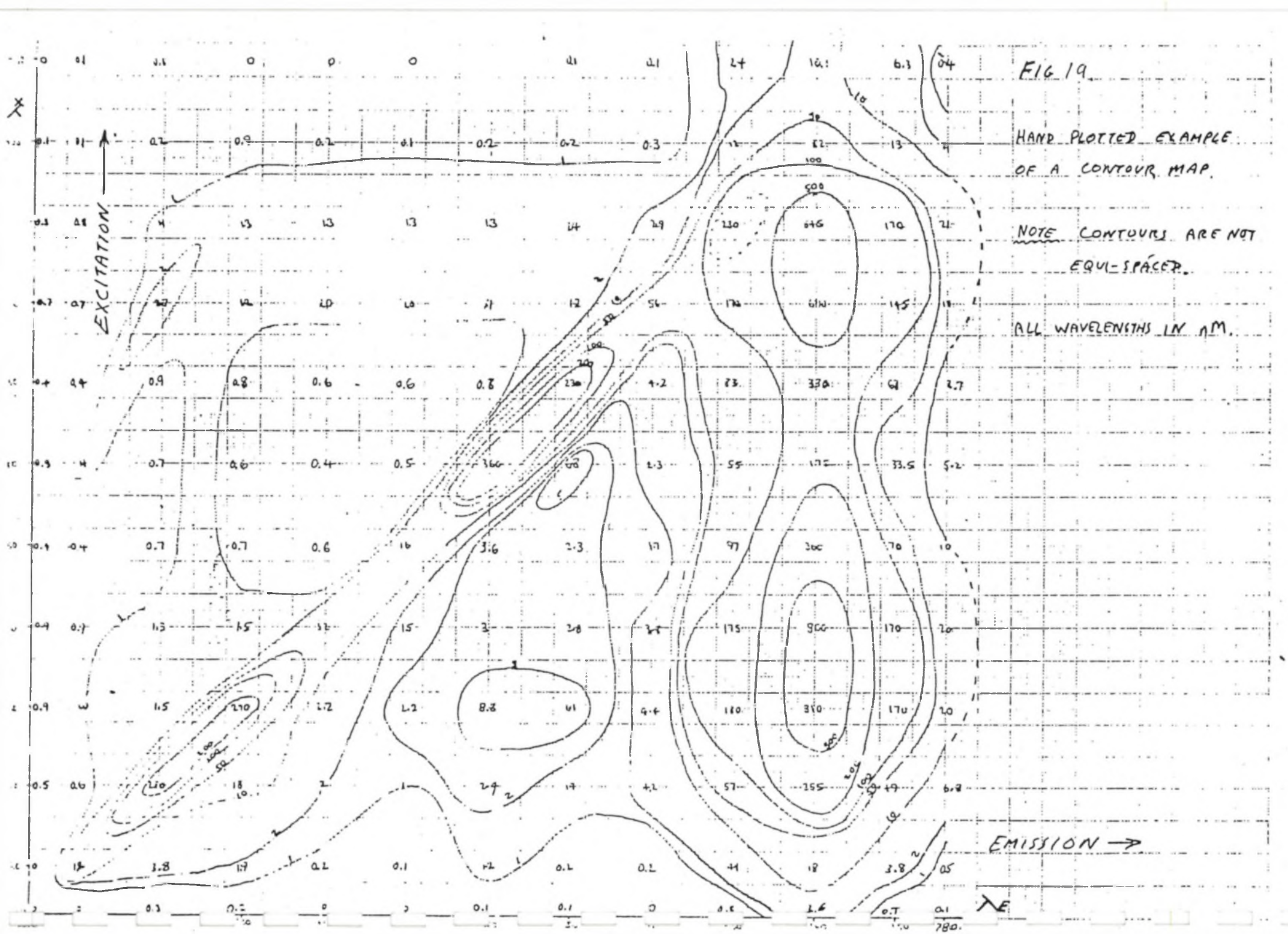


FIG 19.

HAND PLOTTED EXAMPLE OF A CONTOUR MAP.

NOTE CONTOURS ARE NOT EQUI-SPACED.

ALL WAVELENGTHS IN nM.

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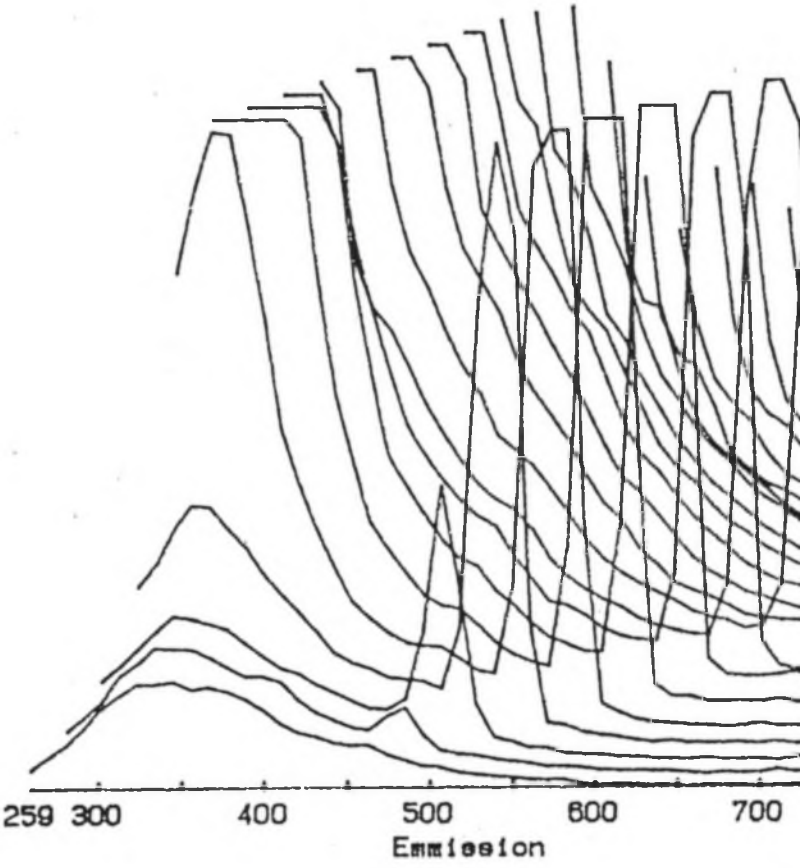
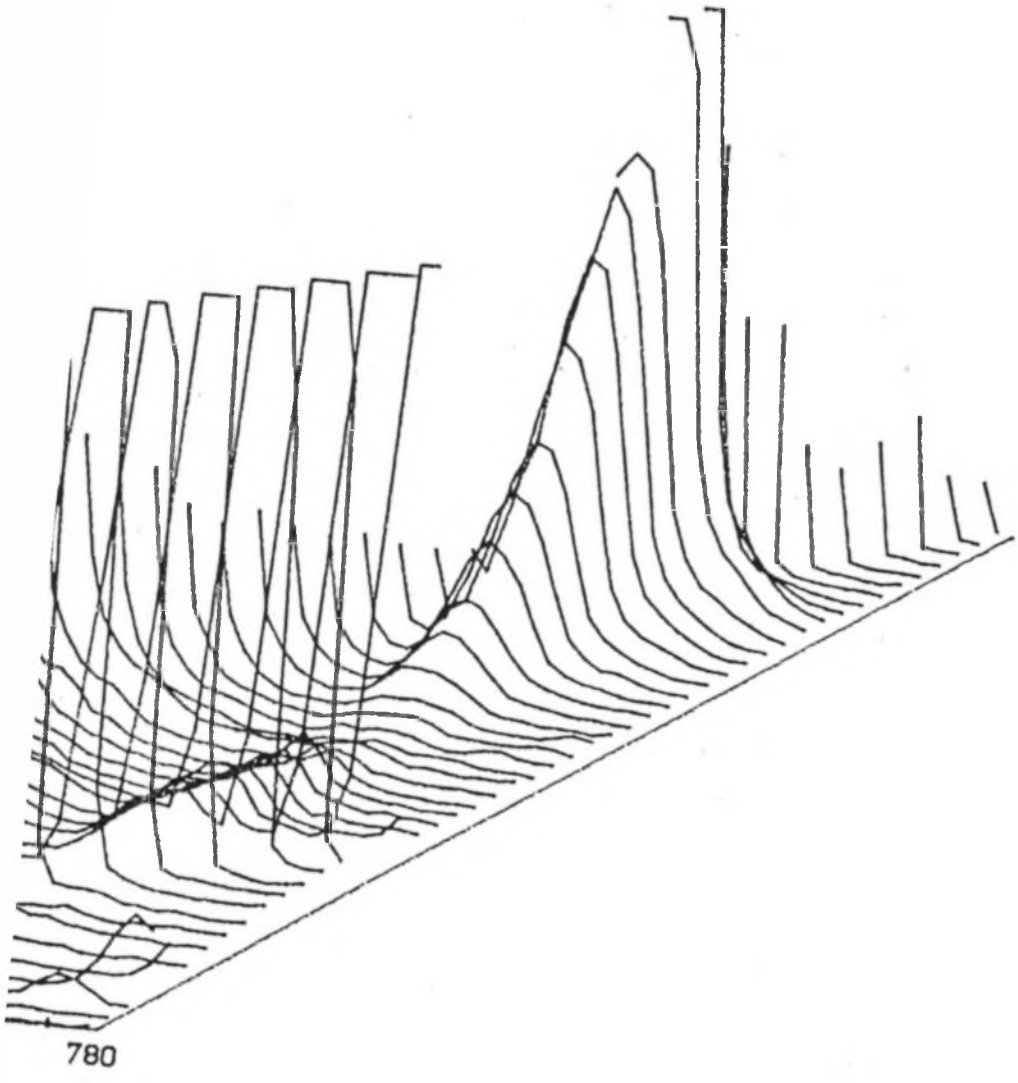


FIG 20



APPENDIX 2
PROGRAM LISTINGS

A copy of this appendix may be obtained from John Hilton or Nigel Hetherington on request.

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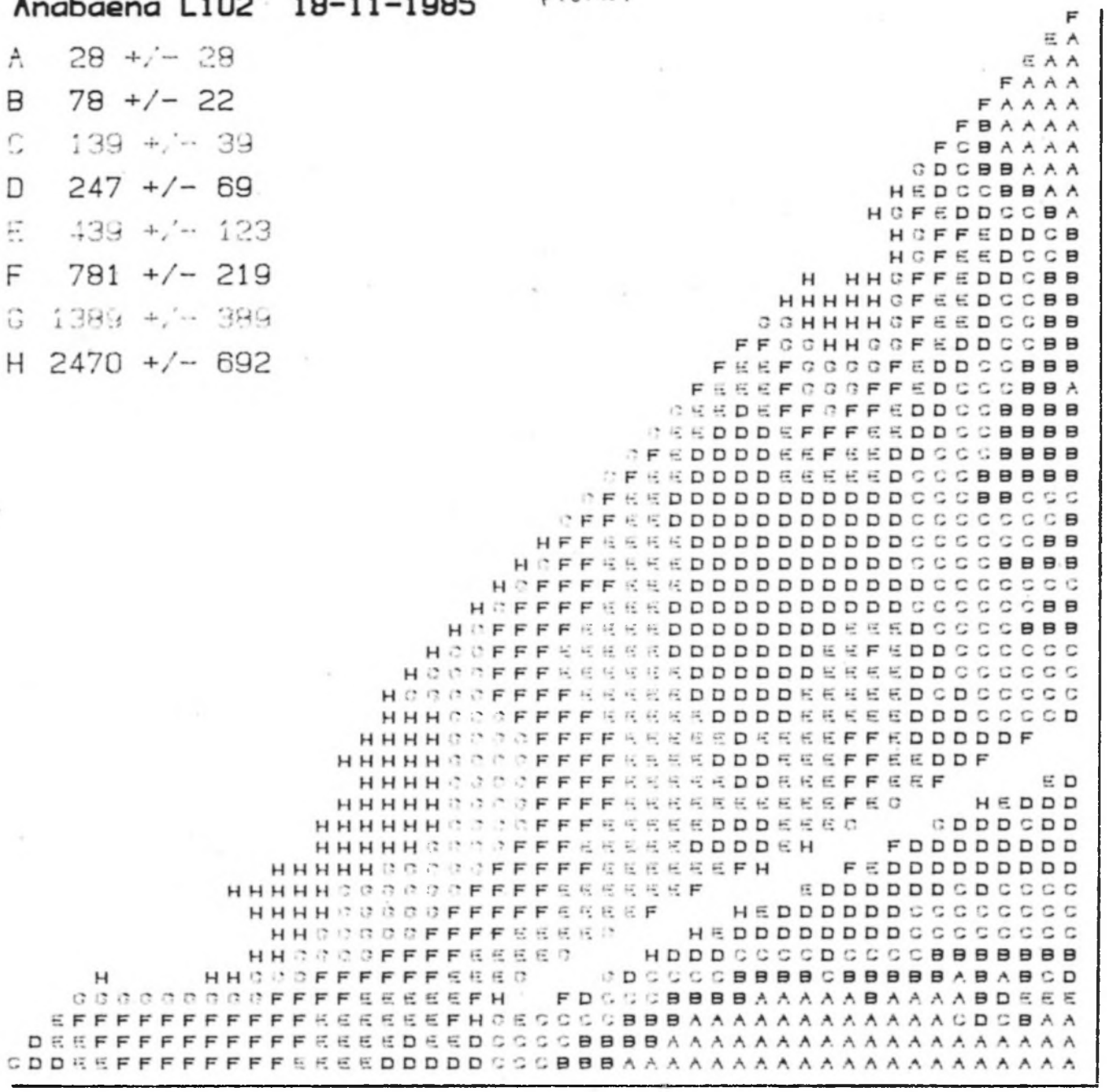
Fig. 21

A 28 +/- 28
B 78 +/- 22
C 139 +/- 39
D 247 +/- 69
E 439 +/- 123
F 781 +/- 219
G 1389 +/- 389
H 2470 +/- 692



Excitation

Emission