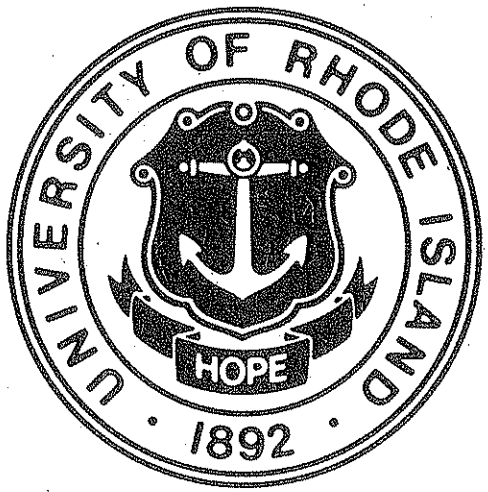
RHODE ISLAND
WATER RESOURCES CENTER



COMPLETION REPORT FY-1983 PROGRAM

RAPID DETECTION AND IDENTIFICATION OF
BACTERIA IN SEWAGE AND NATURAL WATERS
BY MEANS OF TIME-RESOLVED LASER SPECTROSCOPIES

by

W.H. Nelson and J.F. Sperry

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Project No. G867-02

The research on which this report is based was financed in part by the United States Department of the Interior, as authorized by the Water Research and Development Act of 1978 (P.L. 95-467).

Completion Report

Objectives:

The objective of this project has been the investigation of sensitive methods of rapid detection and identification of bacteria. Specifically, we have studied the use of resonance Raman and time-resolved fluorescence spectroscopies.

Related Research:

Previously, we have shown (1) that chromobacteria can be distinguished on the basis of distinctive resonance Raman spectra. Spectra were excited by low-power argon ion laser radiation at 488 nm and are due to the presence of carotenoid pigments. A spectrum of Flavobacterium arborescens is attached (Figure 1). Algae have been studied in similar fashion.

Methods and Procedures:

Using a Raman spectrometer produced commercially by Spex, Inc. were able to laser illuminate and count two types of bacteria under the microscope. Because it was possible to see the laser-excited bacteria while the resonance Raman spectra were being obtained, it was possible to estimate closely the number of bacteria giving rise to a spectrum.

Because of the obvious presence of distinctly different fluorophores in bacteria, we have attempted to assess whether marked differences in fluorescence lifetimes can be used as a means of bacterial investigation. Toward that end, we have studied the fluorescence lifetimes by means of a PRA instrument with 300 ps resolution. In fact, lifetimes between 500 ps and 20 ns could be assessed if no more than three major components were observed at a specific emission wavelength. Emission wavelengths were isolated by means of interferene filters.

Principal Findings and Their Significance:

Spectra attached (Figure 2) of the two types of flavobacteria were the result of scattering from 25-50 organisms. Laser power was low (~5 mw), and moderate background fluorescence was present as interference. Thus, this experiment did not seriously press the potential detection limits. It is clear that the resonance Raman technique can be highly sensitive, and this experiment suggests that both remote detection and detection from mixtures is possible in principle.

In pratice, it is doubted that excitation with visible radiation will allow effective identification of bacteria, simply because most bacteria are not intensely colored and do not give strong resonance Raman spectra in the visible range.

Perhaps a more serious problem is the omnipresent fluorescence due to the cell components or media. A typical bacterial fluorescence emission spectrum is attached. This spectrum of S. epidermidis (Figure 3) excited at 220 nm shows a very broad, relatively featureless bond between 285 and 600 nm due to the contributions of various cell components. On casual examination, problems associated with 285-600 nm resonance Raman excitation are obvious. The fluorescence "background" will obliterate any resonance Raman spectra since the resonance Raman spectra usually are of about the same intensity as the fluorescence, or, an order of magnitude or more, less intense.

The spectrum of S. epidermidis, on the other hand, is even more significant in terms of what it does not show -- fluorescence intensity below 285 nm. This means there should be the possibility of excellent resonance Raman sensitivity associated with cell components which absorb below 285 nm. Since many of the most important cell nucleic acid and protein components absorb in

the 255-290 region or lower, it is apparent that a study based on these will be most attractive. Preliminary studies (3,4) of mononucleotides, tyrosine, tryptophan, DNA, and living cells (Figure 4) show that under very low powers, 2 mw, excitation at 257 nm, very intense, characteristic spectra are obtained without any fluorescence interference.

While resonance Raman studies of DNA especially are expected to provide the basis for rapid identification, fluorescence may provide a better means of assessing cell viability. Fluorescence excitation spectra associated with emission in the 450-550 nm range show marked differences between bacteria which are related to the nature of the bacteria, as well as their history (Figure 5).

The time-resolved fluorecence spectra of several organisms have been studied to determine differences characteristic of specific bacteria. The time-resolved spectra of two of these, Staphylococcus epidermidis and Pseudomonas fluorescens, are attached (Figures 6,7). It is notable that P. fluorescens shows only two components emitting beyond 418 nm (at 1.89 and 7.56 ns, respectively), while S. epidermidis shows three components in that same wavelength range of distinctly different lifetimes (844 ps, 3.88 ns, and 11.46 ns), respectively.

In addition, I wish to note that both resonance Raman and time-resolved fluorescence spectra ultimately will be efficiently excited by a pulsed source in the picosecond time domain. The availability of a reliable, strong tunable source would make possible the simultaneous determination of both types of spectra with separate detectors. Then, a single source and instrument potentially could determine the identity, number, and viability of microorganisms. Remote detection remains a definite possibility, although important questions concern-

ing range and sensitivity must be addressed.

Conclusion:

Resonance Raman and time-resolved fluorescence spectroscopies both show promise as means of detecting identifying and studying bacteria. Because of fluorescence interference in the visible region, and because bacteria are easily differentiated on the basis of nucleic acid content, Raman investigation in the regions associated with strong ultraviolet absorption of nucleic acids is most promising.

Time-resolved fluorescence spectra are notably different for different bacteria. This is very likely due to different degrees of quenching of tyrosine and tryptophan in the proteins of these organisms. Additional work is needed to positively identify the bases for the spectral differences. With a rational basis developed to understand the spectral differences, the time-resolved fluorescence technique has great promise for use in rapid bacterial analysis.

References

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Contents of this publication do not necessarily reflect the views and policies of the United States Department of the Interior, nor does mention of trade names or commercial products constitute their endorsement by the U.S. Government.

FLAVOBACTERIUM ARBORESCENS

(YELLOW - ORANGE)

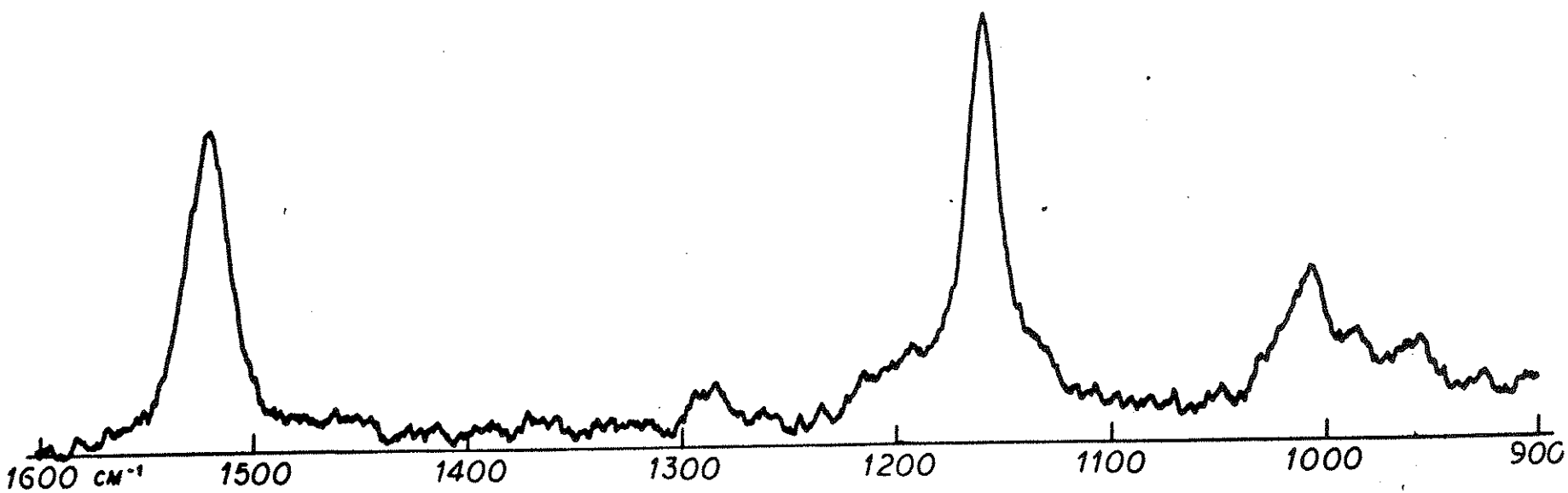


Figure 1

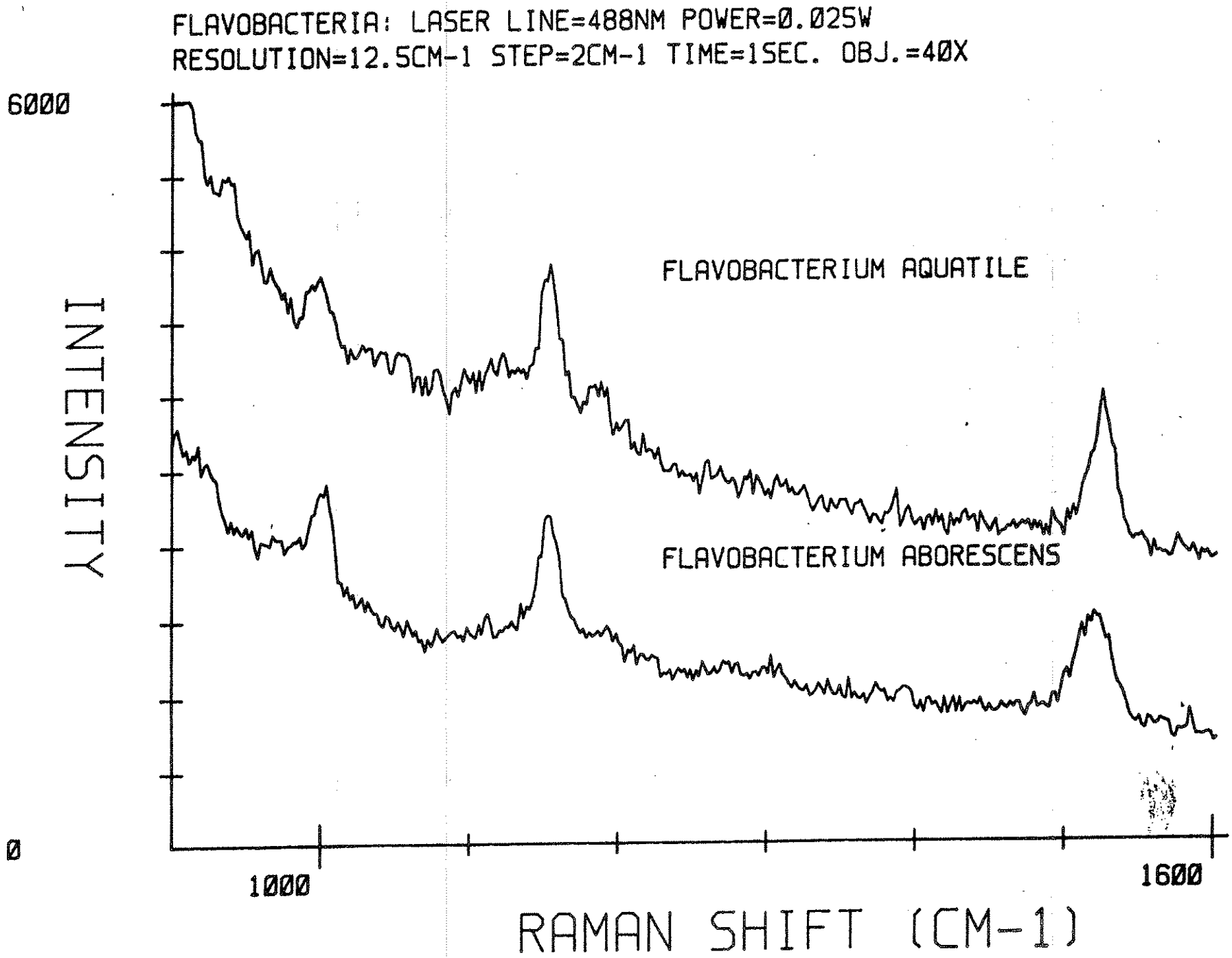

FLAVOBACTERIA: LASER LINE=488NM POWER=0.025W
RESOLUTION=12.5CM-1 STEP=2CM-1 TIME=1SEC. OBJ.=40X
6000
INTENSITY
0
FLAVOBACTERIUM AQUATILE
FLAVOBACTERIUM ABORESCENS
1000
1600
RAMAN SHIFT (CM-1)

Figure 2

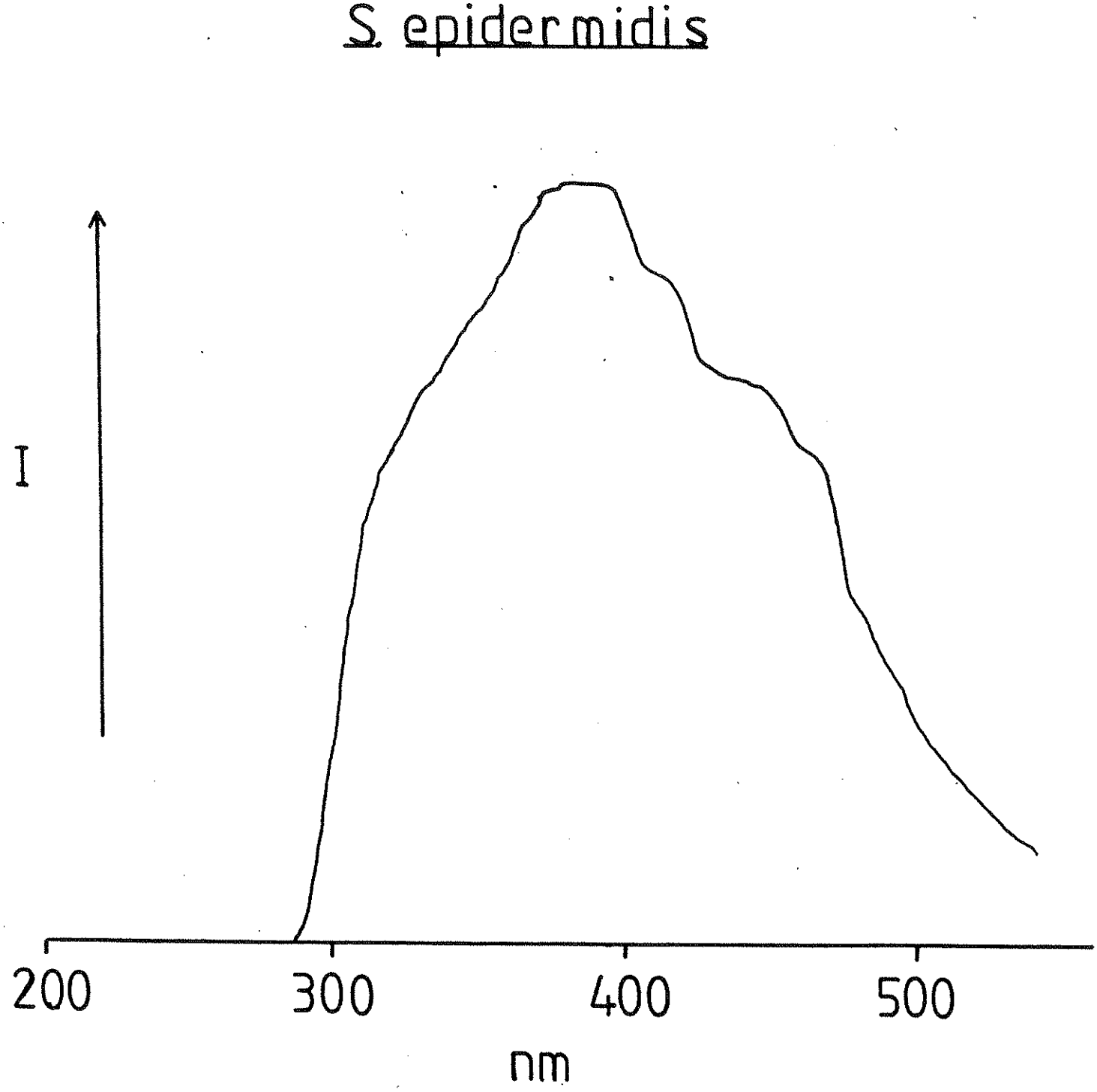
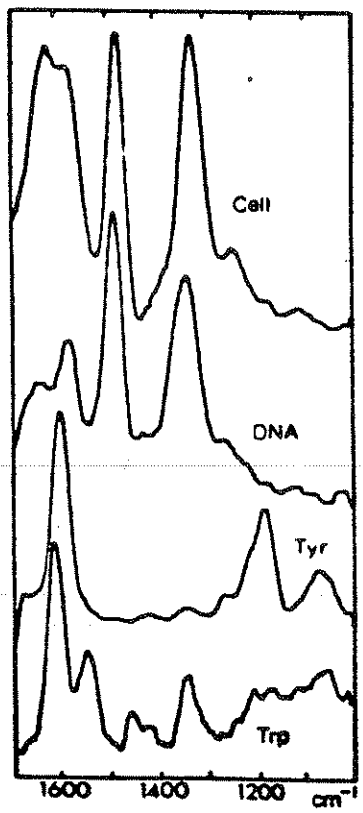
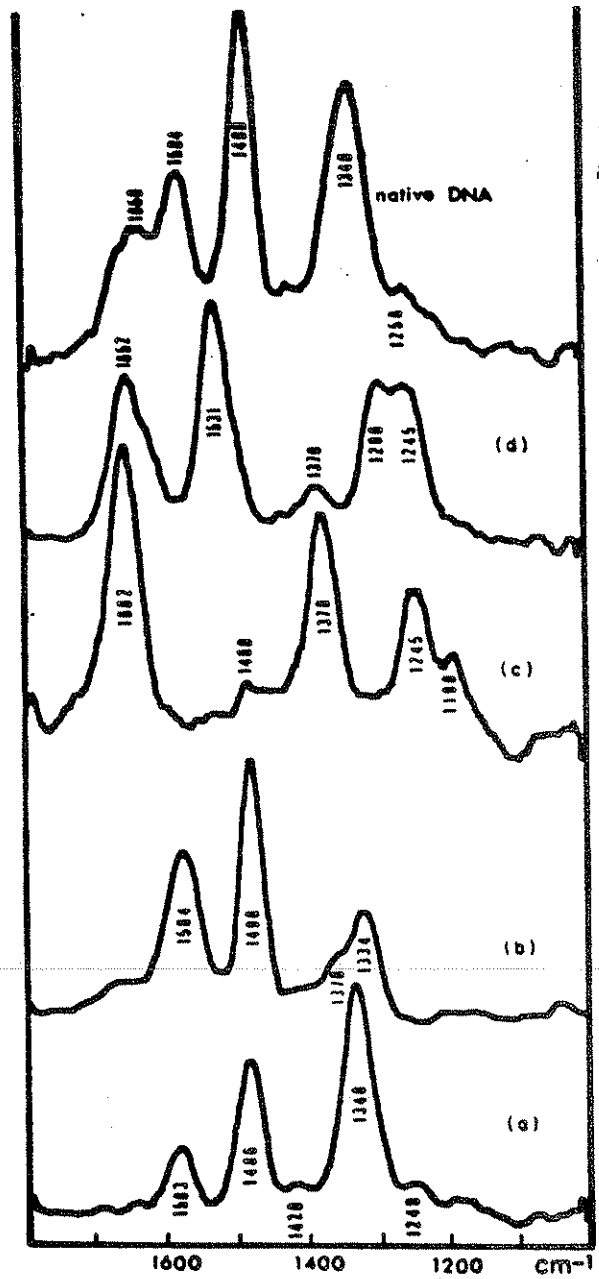


Figure 3



Resonance Raman spectra in the wavenumber shift range of 1000 to 1800 cm^{-1} of *in vitro* DNA 0.1 mol per ml 10^{-2} M NaCl, tyrosine 10^{-3} M pH 7, tryptophane 10^{-3} M pH 7 in neutral aqueous solutions and living cells.



Resonance Raman spectra in the wavenumber shi range of 1000 to 1800 cm^{-1} of the four mononucleotides i neutral aqueous solution; 0.1 mg ml^{-1}, λ exc = 257 nm; (a adenosine monophosphate; (b) guanosine monophosphate; (c thymidine monophosphate; (d) cytidine monophosphate an native DNA in 10^{-2} M NaCl.

Figure 4

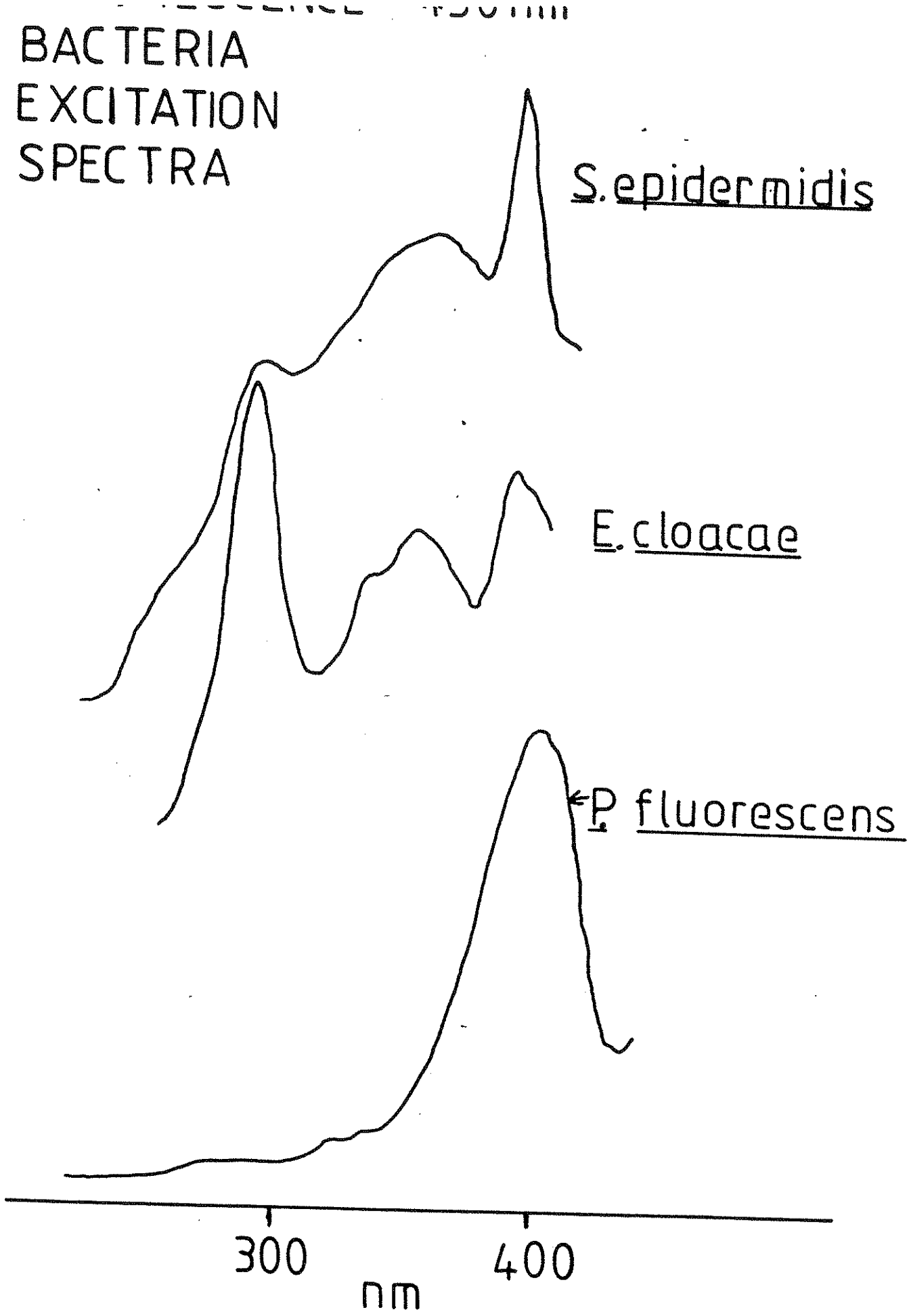

BACTERIA
EXCITATION
SPECTRA
S. epidermidis
E. cloacae
P. fluorescens
300
400
nm

Figure 5

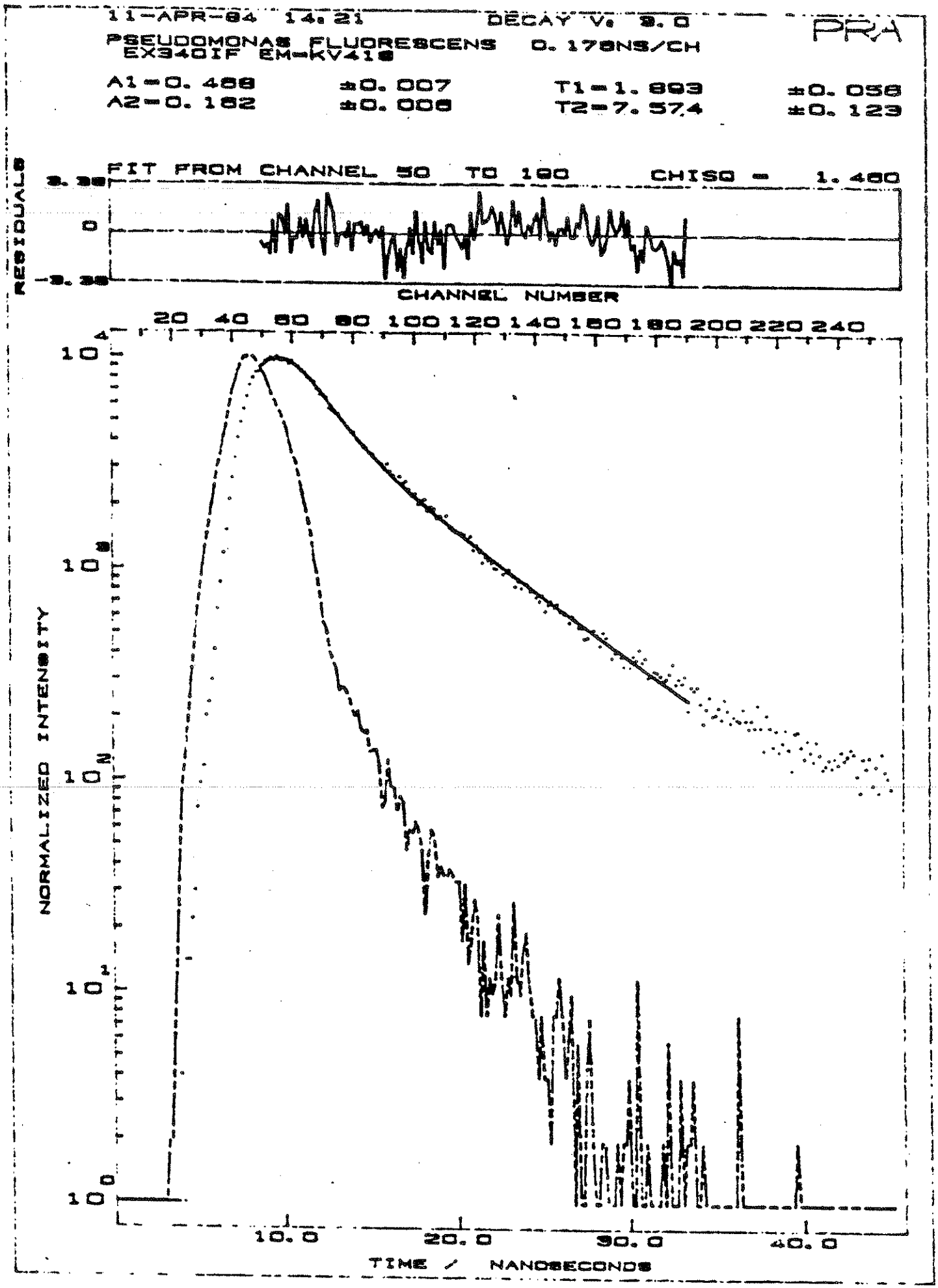

11-APR-84 14:21
DECAY V: 9.0
PRA
PSEUDOMONAS FLUORESCENS 0.178NS/CH
EX340IF EM-KV418
A1=0.468 ±0.007 T1=1.893 ±0.058
A2=0.182 ±0.008 T2=7.574 ±0.123
FIT FROM CHANNEL 50 TO 190 CHISQ = 1.480
RESIDUALS
CHANNEL NUMBER
20 40 60 80 100 120 140 160 180 200 220 240
NORMALIZED INTENSITY
10.0 20.0 30.0 40.0
TIME / NANOSECONDS

Figure 5

11-APR-84 15:31 DECAY V. 3.0

PR-1

STAPHYLOCOCCUS EPIDERMIDIS 0.178NS/CH
EX340IF EM=KV418

A1=0.743	±0.032	T1=0.844	±0.072
A2=0.214	±0.016	T2=3.863	±0.247
A3=0.035	±0.005	T3=11.485	±0.858

FIT FROM CHANNEL 51 TO 256 CHISQ = 1.004