Jerianne Gugel,[1] M.T. (ASCP) and Jay A. Siegel,[2] Ph.D.

Fluorescence of Petroleum Products III. Three-Dimensional Fluorescence Plots of Petrolatum-Based Products

REFERENCE: Gugel, J. and Siegel, J. A., "**Fluorescence of Petroleum Products III. Three-Dimensional Fluorescence Plots of Petrolatum-Based Products,**" *Journal of Forensic Sciences*, JFSCA, Vol. 33, No. 6, Nov. 1988, pp. 1405-1414.

ABSTRACT: A high degree of association between known and unknown specimens of petrolatum-based lubricants, which occur as evidence in criminal sexual assault cases, can be demonstrated by using three-dimensional fluorescence spectroscopy (3-D fluorescence). Fifteen petrolatum-based products, five mock cases, and two cases provided by the Michigan State Police Crime Laboratory are analyzed and compared using this technique. Controls are run to eliminate interfering substances and to insure proper technique. Three-dimensional plots of excitation, emission, and synchronous excitation fluorescence spectra are collected. A computerized comparison of the resultant plots is conducted to determine if any differences occur between the plots. The results of these analyses suggest that this technique has great potential as an analytical tool, allowing the analyst to determine whether or not two petrolatum-based products could have a common source.

KEYWORDS: criminalistics, petroleum products, criminal sex offenses, spectroscopic analysis, three-dimensional fluorescence spectroscopy

In recent times, it has become increasingly important to characterize and determine the sources of lubricants encountered as evidence in criminal sexual assault cases. These lubricants, petrolatum-based jellies and hair preparations, are often available at the scenes of rapes and forced sodomies and are employed by the assailant for use in attacking the victim. In cases in which the victim has been assaulted and a petrolatum has been used, it is often possible to retrieve some of the petrolatum for analysis by using oral, vaginal, and anal swabs, collecting lubricant-stained clothing or bedding or, if it has been left at the scene, the container of lubricant used in the assault. Retrieving the container of petrolatum is extremely important because it serves as a known source with which the unknown specimen, taken from the victim, can be compared. In addition, the container may contain the assailant's latent prints, which can directly link the individual to the scene of the crime.

Methods that are presently employed in analyzing this type of evidence, with varying degrees of success, include packed-column gas chromatography, infrared spectrometry, simple fluorescence spectroscopy, and combinations of these techniques. Capillary gas chromatography has also been used with some success in the analysis of these petrolatum products.

Received for publication 2 Nov. 1987; revised manuscript received 29 Jan. 1988; accepted for publication 29 Jan. 1988.

[1]Forensic scientist, Michigan State Police, Forensic Science Division, East Lansing, MI.
[2]Associate professor, School of Criminal Justice, Michigan State University, East Lansing, MI.

Another technique which has proven beneficial in the analysis of petroleum-based products is three-dimensional (3-D) fluorescence spectroscopy [*1*].

Petrolatum is a purified unctuous, semisolid mixture of hydrocarbons varying in color from nearly colorless to amber and containing approximately 16 to 32 carbon atoms per molecule. It is often employed in the preparation of pharmaceutical ointments [*2*].

Petrolatum, in that it is derived from petroleum, exhibits significant fluorescence as a result of the presence of polynuclear aromatic hydrocarbons (PAHs). Determination as to the different brands of petrolatums can be made based upon the fluorescence properties and the relative amounts of PAHs contained within each product. Differences between lot numbers of the same type of product may possibly be determined as well. Hence, the fluorescence properties of petrolatums may be thought of as unique markers for those products and, as a result, may provide the analyst with a means to determine, by direct comparison, whether or not two specimens could have had a common source.

Using the 3-D fluorescence technique and Siegel's computer subtraction program, enough spectral information about any petroleum-based product can be generated to distinguish between two closely related specimens [*3*]. This technique involves the collection of a number of fluorescence spectra. Excitation, emission, and synchronous excitation spectra are obtained under different conditions. Each plot consists of 30 spectra; thus, a contour plot having a "mountain-range" appearance results. Two different contour plots of excitation spectra can be produced, as well as two emission and two synchronous excitation plots. The two different plots produced consist of views from high and low wavelengths of the fluorescence of the specimen. Siegel's computer program allows for the comparison of two different plots by subtracting one plot from the other, spectrum by spectrum [*4*].

This paper is the third in this series and involves a study of petrolatum-based products, their fluorescence properties, and comparisons of those properties. The purposes of this study are three-fold:

(1) to determine the range of fluorescence for petrolatum-based products used in criminal sexual assault cases,

(2) to determine if different brands of petrolatums can be differentiated using 3-D fluorescence spectroscopy, and

(3) to test further Siegel's computer comparison program using blind testing on several petrolatum products.

Experimental Methods

In this study, 15 different petrolatum-based products were analyzed and used as standards. This number was chosen because it was believed to represent adequately the range of products of this type presently available. The petrolatums are thick, gel-like products which, in this state, are unsuitable for fluorescence analysis. For this reason, each specimen was taken directly from its respective container and diluted to the optimum concentration (determined empirically to be 1.0 mg/mL) in spectro-grade cyclohexane (Burdick-Jackson). The 15 specimens studied were the following:

(1) A&D Ointment,
(2) Vick's Vapo Rub®,
(3) Dark & Lovely® Protein Hair Dress/Conditioner,
(4) Posner Bergamot Conditioner and Hair Groom,
(5) Ultrasheen® Conditioner & Hair Dress for Extra Dry Hair,
(6) Posner Coconut Oil Conditioner,
(7) Ultrasheen Conditioner & Hair Dress (regular),
(8) Sulfur-8® Hair & Scalp Conditioner (original formula),
(9) Vaseline® Petroleum Jelly (Lot 1),
(10) Vaseline Petroleum Jelly (Lot 2),

(11) Meijer Petroleum Jelly,
(12) No Brand Pure Petroleum Jelly (generic),
(13) Alberto-Culver TCB Concentrated Hair & Scalp Conditioner,
(14) Proline Hair Food, and
(15) Blue Magic® Bergamot Hair & Scalp Conditioner.

The cyclohexane was determined not to contribute any significant fluorescence to the spectra and thus did not interfere in the analysis of any of the petrolatums. The authors' previous experience with fluorescence of petroleum products has been that they are prone to leave some residues behind in the glassware used in making up the solutions. To guard against any chance of this occurring and to minimize the chance of any residual fluorescence from such residues, a specific washing sequence was used: cyclohexane followed by acetone, distilled water, concentrated nitric acid, and then distilled water again. The glassware was then dried in an oven at 60°F (15.5°C).

In the analysis of the five mock cases and the two actual cases, the lowest concentration of petrolatum yielding the most information was used. Unlike the set concentration of 1 mg/mL for the standards, the concentration of the petrolatum in the mock and actual cases was not known. In any case, the maximum fluorescence in each specimen was normalized.

For the five mock cases, a few of the standard petrolatums were selected for the blind study. A small amount of the selected petrolatum was placed in a test tube directly from the container. The specimens were dissolved in cyclohexane as in the case of the standards.

The two actual cases obtained from the Michigan State Police Crime Laboratory required more preparation, since the specimens were obtained from vaginal swabs and panties taken from the victims. These specimens were extracted in cyclohexane, centrifuged, and filtered to eliminate any interfering particles or substances that might be present. The specimens were then evaporated to dryness and reconstituted in cyclohexane at the optimum concentration, again determined empirically. Each specimen was prescanned in the spectrofluorimeter to determine the wavelengths of maximum excitation and emission. The parameters determined by the prescan were then used to obtain normal excitation, emission, and synchronous fluorescence plots of the petrolatums. Based on these results, conditions were then selected to obtain the 3-D plots.

Instrumentation and Software

The spectrofluorimeter used in this study was a Perkin-Elmer Model LS-5 connected to a Model 3600 Data Station. The excitation and emission slits were set to 5 nm. Perkin-Elmer PECLS II software was used to collect spectral data, and Perkin-Elmer's proprietary graphics plotting software (PLOT), operating under Perkin-Elmer's proprietary disk operating system (PETOS), was used to plot the spectral data. In addition, Perkin-Elmer's proprietary total fluorescence data collection software (LU3D) and Siegel's synchronous fluorescence total fluorescence data collection program written in Perkin-Elmer's OBEY language (SY3D) were used to collect the 3-D spectra, and Siegel's DF3D program was used to compare, by computer subtraction, the 3-D plots.

Results and Discussion

All of the petrolatum specimens exhibit their fluorescence peaks in the same general region. For excitation and synchronous excitation, the range is 200 to 350 nm, and for emission, the range is 280 to 450 nm. After careful examination of the 3-D plots for all 15 specimens, the synchronous excitation plots were found to exhibit the most discrimination between specimens and therefore were used for comparison purposes for the remainder of the study.

The 3-D synchronous plots for one of the original fifteen specimens is shown in Fig. 1.

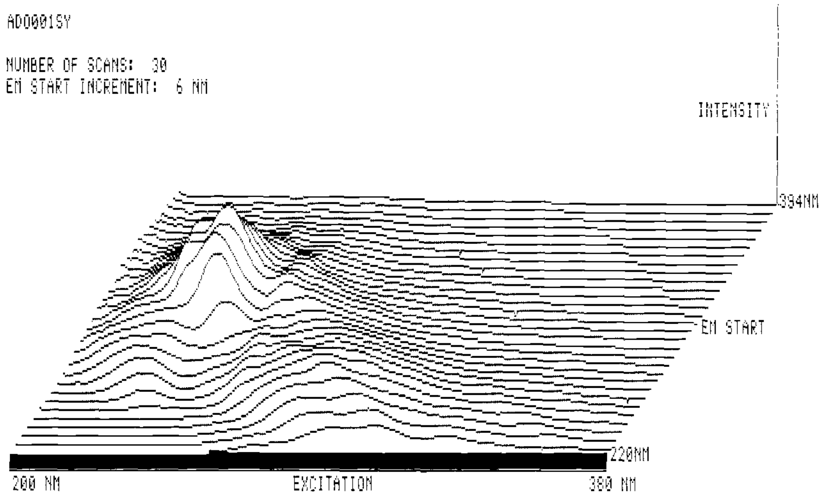

FIG. 1—*Synchronous 3-D plot of A&D Ointment.*

Siegel's 3-D computer subtraction program is capable of graphically depicting whether or not two 3-D plots are different from one another. Without the aid of this program, this type of comparison done visually is extremely difficult and, most likely, insufficient to make a definitive determination. Siegel's subtraction program was employed in the study of the five mock cases and the two actual cases covered in this study.

Five Mock Cases

A total of ten specimens of petrolatums, selected from those previously run, were presented in pairs to the analyst. Three-dimensional fluorescence plots were generated for each specimen. The subtraction program then obtained resultant plots from the subtracted pairs. The results are shown in Figs. 2 to 6. In theory, the resultant 3-D subtracted plot should be planar if the two specimens have a common source (with the exception of noise appearing in the range of 200- to 230-nm excitation). If the resultant 3-D subtracted plot has a "hilly" appearance, then one would conclude that the specimens had different sources.

The following results were obtained from the blind study: Specimens A (Fig. 2) and D (Fig. 5) were determined to be associated with the other member of the respective pairs, and the members of the pairs could have had a common source. Paired Specimens C (Fig. 4) and E (Fig. 6) were determined to have had different sources. The specimens in Case B (Fig. 3) were determined to be inconclusive but probably of different sources. The actual composition of the specimens are given below.

A: Meijer Petroleum Jelly—Meijer Petroleum Jelly
B: Meijer Petroleum Jelly—No Brand Pure Petroleum Jelly
C: Posner Bergamot Conditioner & Hair Groom—Ultrasheen Conditioner & Hair Dress (regular)
D: Sulfur-8 Hair & Scalp Conditioner—Sulfur-8 Hair & Scalp Conditioner
E: A&D Ointment—Vick's Vapo Rub

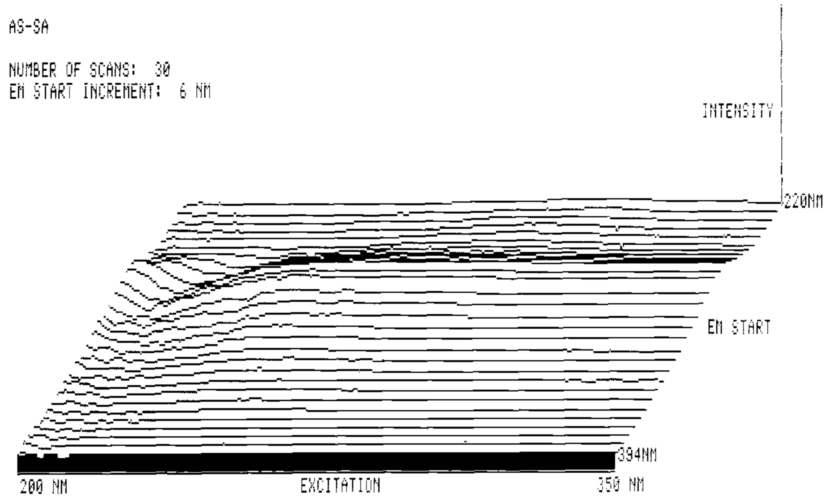

FIG. 2—*3-D plots of Mock Case A.*

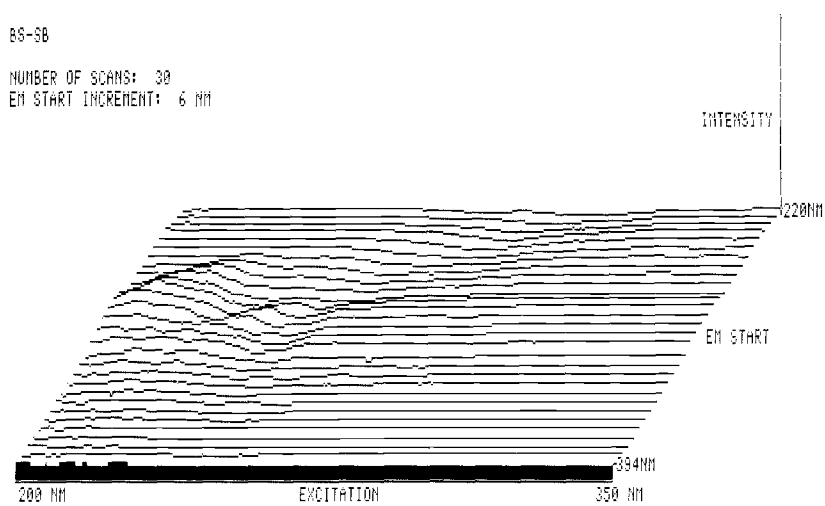

FIG. 3—*3-D plots of Mock Case B.*

As is evident from the subtracted 3-D plots, those pairs having different sources had obvious areas of net fluorescence and were easily distinguishable from those plots which were flattened, since the fluorescence intensity here is essentially zero. In the case of Mock Case B, the results indicate that both specimens probably had a different source, but the results were not as dramatic as in the other cases. This is not surprising, though, since the two specimens were both petroleum jellies. This result indicates that in the case of pure petroleum jellies, differentiation between brands may not be possible.

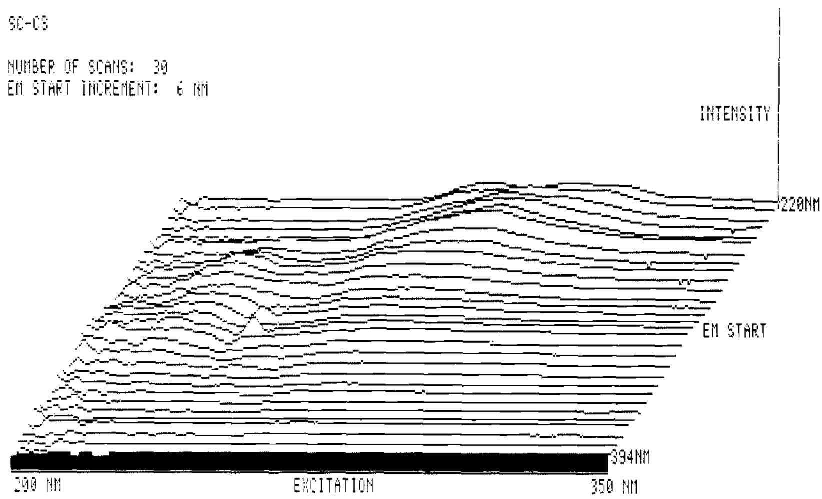

FIG. 4—*3-D plots of Mock Case C.*

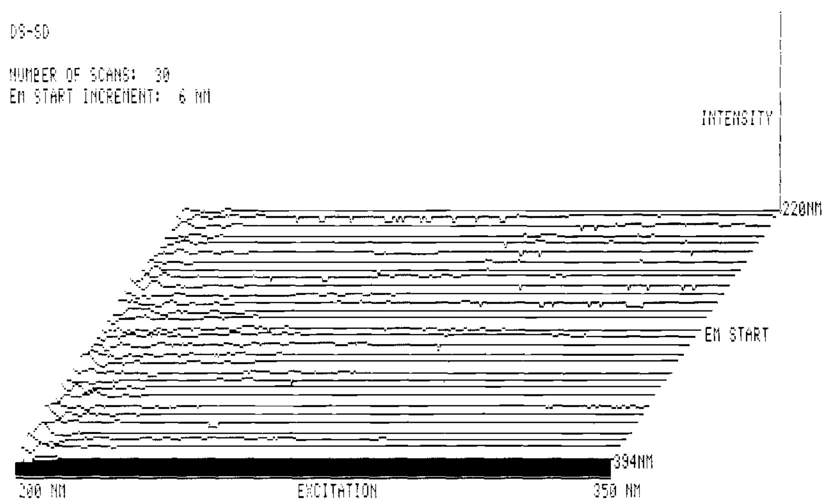

FIG. 5—*3-D plots of Mock Case D.*

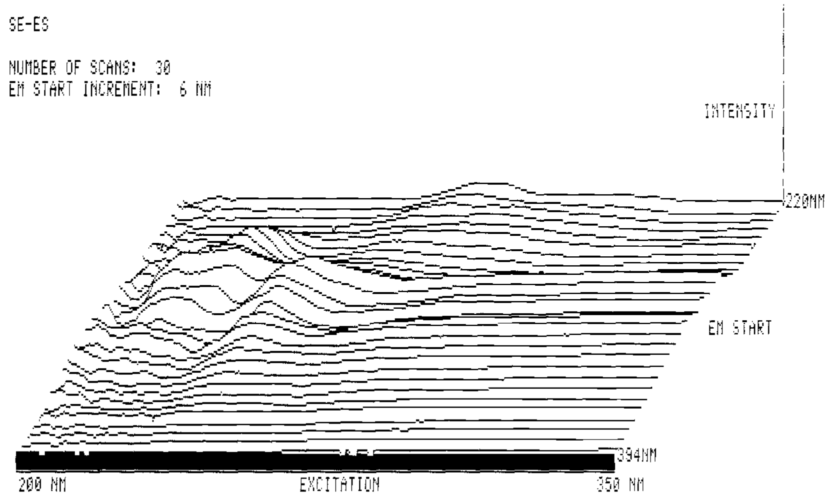

FIG. 6—*3-D plots of Mock Case E.*

Two Actual Cases

The following results were obtained as a result of the two actual cases analyzed which are shown in Figs. 7 and 8: Case 1: known and unknown could have had a common source (Fig. 7) and Case 2: known and unknown could have had a common source (Fig. 8).

The flattened appearance of the resultant 3-D subtracted plot in Case 1 (Fig. 7*c*), indicates a high degree of association between the specimens and suggests that the two specimens are highly associated. In Case 2 (Fig. 8*c*), the result is similar although the plot is not as planar as in Case 1. Nonetheless, the differences that are present do not seem as significant as those found in the studies of the standards, and a high degree of association is indicated.

Summary

Three-dimensional stacked plot fluorescence spectroscopy has been evaluated for its ability to differentiate between brands of petrolatum-based products. The technique is capable of providing the analyst with the total fluorescence of a specimen. This technique has proven beneficial in its ability to compare known and unknown specimens and in determining the degree of association between such specimens. An area for further study, suggested by these results, concerns differentiation between different brands of pure petroleum jellies using 3-D fluorescence.

As a result of this research, Siegel's 3-D comparison program has been tested using additional blind trials and has been applied to the analysis of petrolatums. Once again, the program has proven successful in aiding the analyst in determining the degree of association between two similar specimens.

Acknowledgment

The authors would like to express their appreciation to the Michigan State Police Forensic Science Division for the evidence from the two sexual assault cases.

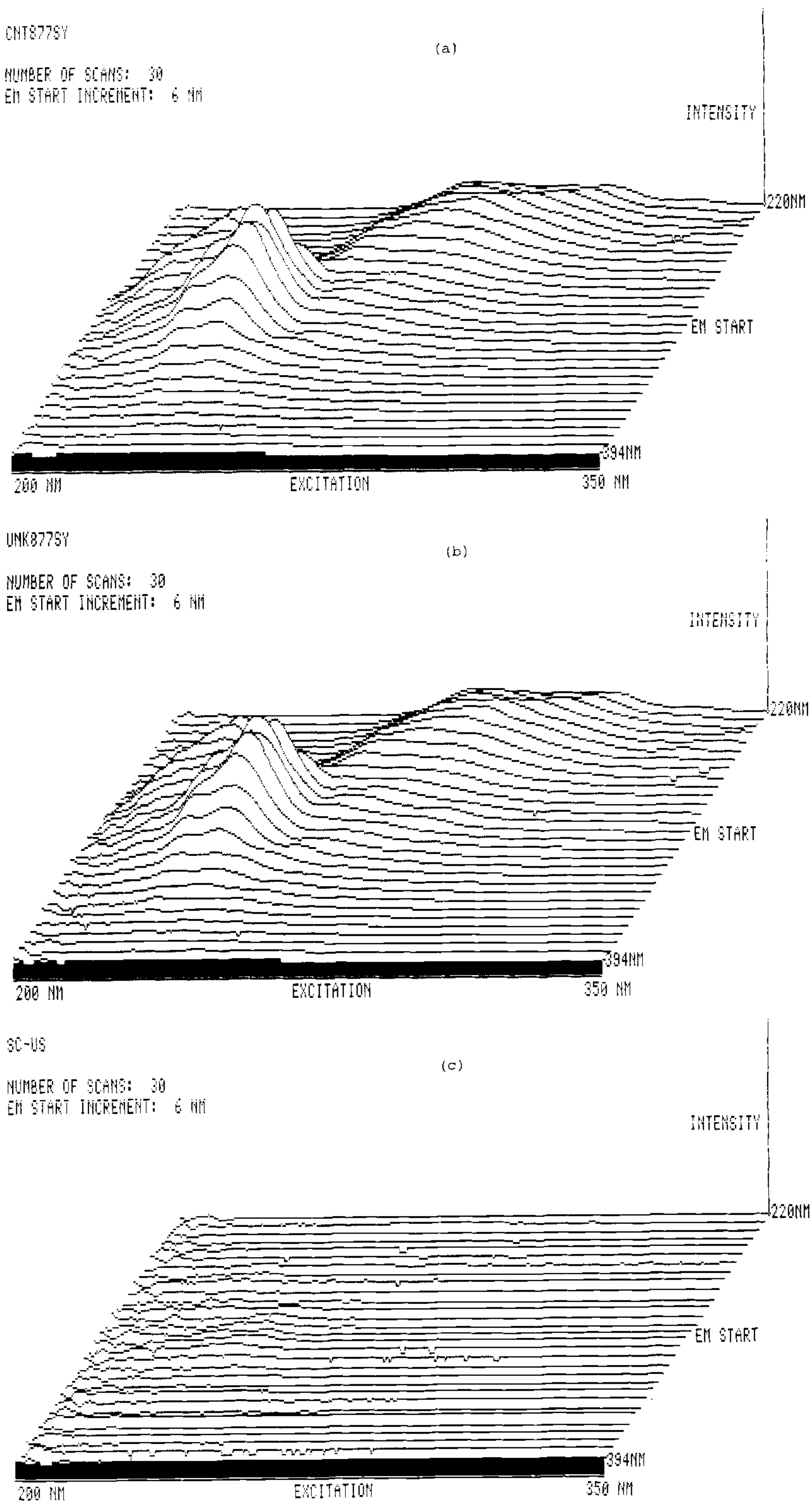

FIG. 7—*Synchronous and subtracted 3-D plots of Actual Case 1.*

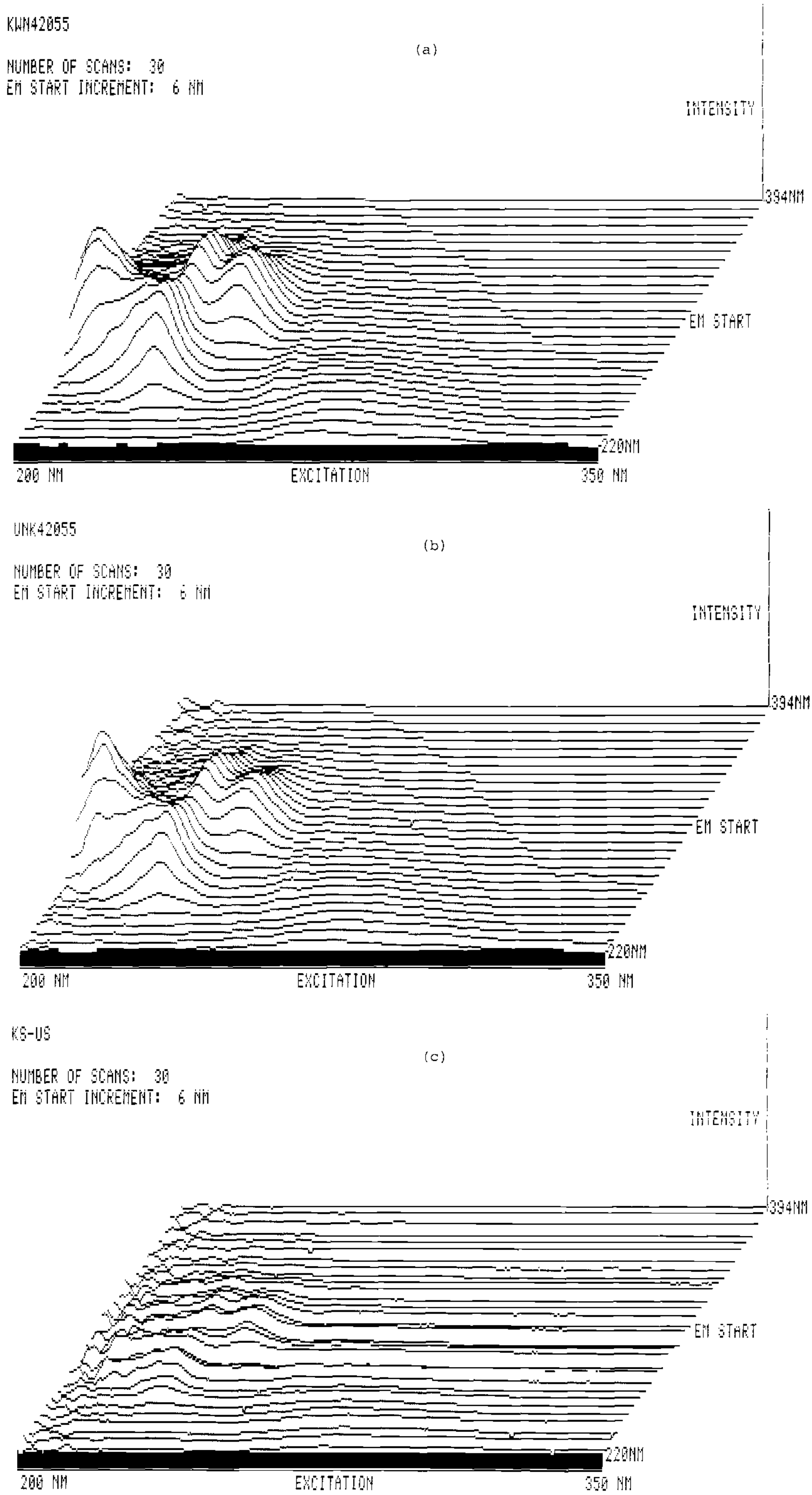

FIG. 8—*Synchronous and subtracted 3-D plots of Actual Case 2.*

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Address requests for reprints or additional information to
Jay A. Siegel, Ph.D.
Michigan State University
School of Criminal Justice
560 Baker Hall
East Lansing, MI 48824-1118