TECHNICAL NOTE

Louise M. Davies,[1] *M.Sc.; Nia E. Jones,*[1] *M.Sc.; John S. Brennan,*[1] *D.Phil.; and Simon K. Bramble,*[1] *Ph.D.*

A New Visibly–Excited Fluorescent Component in Latent Fingerprint Residue Induced by Gaseous Electrical Discharge

REFERENCE: Davies LM, Jones NE, Brennan JS, Bramble SK. A new visibly–excited fluorescent component in latent fingerprint residue induced by gaseous electrical discharge. J Forensic Sci 2000;45(6):1294–1298.

ABSTRACT: A technique that exposes fingerprint residue to a gaseous electrical discharge in nitrogen followed by treatment with ammonium hydrogen carbonate vapors to produce fluorescence is investigated. Particular attention is made to fluorescence observed via laser illumination at 514 nm. Insight into the nature of the fluorescent components is achieved through the use of thin-layer chromatography (TLC) of fingerprint residue. Results reported indicate the fluorescence observed is from previously non-fluorescent fractions of the fingerprint residue, and TLC results point towards lipid derivatives as a possible source of the fluorescence.

KEYWORDS: forensic science, fingerprints, thin-layer chromatography, gaseous electrical discharge, fluorescence, lipid

Laser-induced fluorescence continues to provide law enforcement agencies with a powerful method for the location of latent fingermarks (1). The success of this technique relies on the material deposited by a fingertip containing fluorescent compounds. However, it is thought that the tendency of individuals to deposit fluorescent fingerprints varies not only between individuals but also within a single individual over time. In order to increase the effectiveness of latent fingerprint location using natural fluorescence, a greater understanding of both the residue chemistry and photophysics is essential. As a result there have been several studies either attempting to identify the fluorescent compound(s) or to increase the fluorescence yield (2–8). An interesting approach to the latter issue was proposed by Meylan et al. (8). They applied a gaseous electrical discharge technique used previously for visualizing organic compounds on thin-layer chromatography (TLC) plates (3,4). The method involved placing a fingerprint downstream of an electrical discharge through gaseous nitrogen followed by treatment with ammonium hydrogen carbonate vapors. An increase in visible fluorescence was reported on non-porous substrates such as metal, glass, aluminum, and porcelain when visualized under 340 nm excitation. Meylan et al. (8) did not investigate the nature of the natural or induced fluorescent components. Since the initial work, there has been no further developments of this promising technique despite its potential for developing fingerprints that are only partially resolved by inherent fluorescence. In particular, it was this potential of the method that stimulated the work presented here.

Although Meylan et al. (8) stated the best excitation was 340 nm, it was decided to assess the technique under 514 nm since argon-ion laser illumination is the primary evidence location tool currently used within the Forensic Science Service (9). After establishing the success of the technique under 514 nm illumination, the aim was to determine whether the increase in the fluorescence signal observed after electrical discharge treatment was from an increase in fluorescence yield from pre-existing residue component(s) or from a new compound(s).

Observation of the fluorescent components within fingerprint residue relies on a robust TLC method that is able to separate three or more fluorescent compounds from fingerprint residue (10). It is this method that is used to monitor the effects of the electrical discharge on the existing fluorescing component(s) present within fingerprints.

Experimental Strategy

The experimental work was divided into two experiments. The first was designed to establish whether the latent fingerprint fluorescence observed after electrical discharge treatment was an increase in natural fluorescent yield or an induction of another component of fingerprint residue to fluoresce. This was achieved by separating the fingerprint residue by TLC and then discharging the separated components. As the electrical discharge chamber was unable to accommodate the developed TLC plate it was necessary to cut the plate into sections. After treatment with the electrical discharge system the plates were subjected to one or more of the visualization treatments described in the experimental procedure.

The second experiment was designed to check that the induction of fluorescence within the fingerprint residue was not dependent upon initially separating the residue by TLC. Thus, the first experiment was essentially run in reverse; that is, electrical discharge treatment of the fingerprint was carried out prior to the separation of the residue by TLC.

[1] The Forensic Science Service, Metropolitan Laboratory, 109 Lambeth Road, London, SE1 7LP, UK.

Received 10 Sept. 1999; and in revised form 9 Nov. 1999, 10 Jan. 2000; accepted 17 Jan. 2000.

Experimental Procedure

TLC Separation of Fluorescent Components from Fingerprint Residue

The TLC method developed by Jones et al. (10) was used to separate the fluorescent components of fingerprint residue. Donor fingerprints were deposited approximately 1 cm from the bottom of Merck aluminum or glass-backed high-performance thin-layer chromatography (HPTLC) plates (silica gel 60, pre–coated, non–fluorescent, 10 cm × 20 cm). Six male subjects of Caucasian origin (aged between 25 and 35 years) provided sebum-rich fingerprints by rubbing their fingers over their forehead and nose prior to deposition. Plates were stored under ambient laboratory conditions (20 to 30°C, 30 to 45% relative humidity, and fluorescent strip lighting) for 24 h prior to development. A chloroform/methanol solvent system (4:1) was used to develop the TLC plates. For a more detailed description of the TLC method refer to the work of Jones et al. (10).

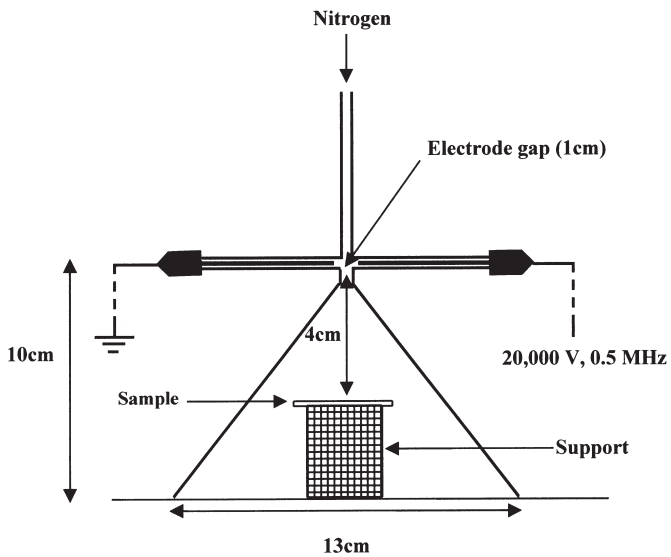

FIG. 1—*Electrical discharge chamber.*

Gaseous Electrical Discharge Treatment

An electrical discharge apparatus based on the design of Meylan et al. (8) was built in-house. A schematic of the electrical discharge apparatus is shown in Fig. 1. The electrical charge was supplied by a high-frequency tester capable of a maximum 20 000 V, 0.5 MHz (Model BD-40, Electro-Technic Products, Chicago). This was connected to a modified glass funnel containing two steel electrodes positioned 1 cm apart at right angles to the flow direction of nitrogen gas to be activated (flow rate of 3 to 6 L/min). The flow of nitrogen through the high voltage spark produced between the two electrodes resulted in a purple/blue jet of activated gas. Optimization of the electrical discharge procedure was achieved by visual inspection of the increase in fluorescent yield. This was determined under reproducible conditions of laser power and viewing filter. Trials established that the largest increase in fluorescence was routinely gained at a distance of 4 cm between the section of TLC plate and discharge for a period of 20 min. After the electrical discharge treatment the sample was placed on a hot plate at approximately 130°C with 2 g of ammonium hydrogen carbonate (NH_4HCO_3). An inverted beaker was used to cover the hot plate and contain the ammonium hydrogen carbonate vapors. It was found that 10 min gave sufficient fuming exposure.

The time between discharge and fuming steps did not appear to be vital and no obvious deterioration of the fluorescence after treatment was noticed over several days, although this was not studied closely. In order to visualize the separated components after electrical discharge treatment three different techniques were used:

(1) Fluorescence—The TLC plate was illuminated at 514 nm with an irradiance of 250 Wm^{-2} from an argon ion laser (Spectra Physics, Model 20–85). The resultant fluorescence was observed through laser goggles fitted with 550 nm long pass filters (Lase-R Shield Inc., Model GU1). Refer to Fig. 2 for the transmission spectrum of the goggles.

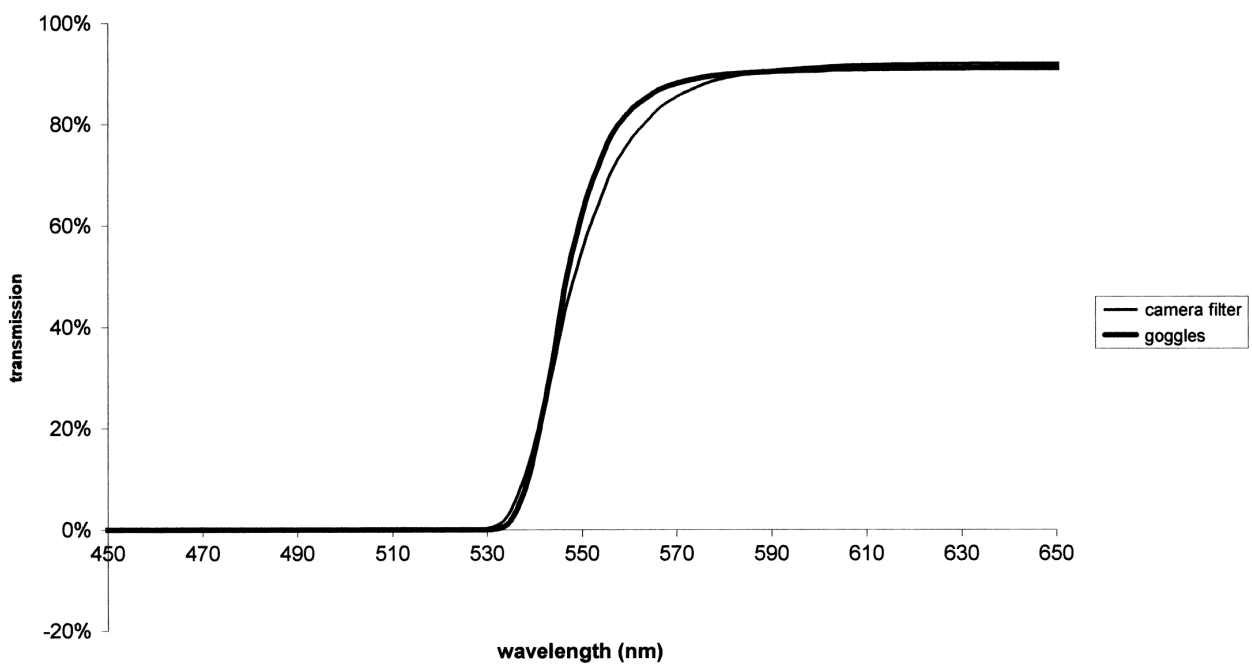

FIG. 2—*Transmission spectra of the laser safety goggles and the camera filter.*

(2) Charring—Merck glass-backed high-performance TLC plates (silica gel 60, pre–coated, non–fluorescent, 10 cm × 20 cm) were used for charring. TLC plates were dipped into an aqueous solution of 10% copper sulfate, 8% phosphoric acid for 10 s and placed on a hot plate at 200°C for 2 to 4 min. Lipid material chars a brown color.

(3) Ninhydrin Reagent—TLC plates were dipped in a ninhydrin solution (0.5% in CFC 113) and then placed in a humidifier (Vindon Scientific Ltd.) at 75°C, 75% relative humidity for approximately 10 min. Ninhydrin reacts with amino acids to form Ruhemann's purple (11,12).

Reagents

Analytical reagent grade solvents were used throughout and purchased from BDH Chemical Company.

Image Capture

Images of fluorescence were captured using a Kodak DCS 460D digital camera. The exposure was 30 s at f stop 11. The plates were "painted" with the laser illumination to expose the entire area to the irradiation. The camera was fitted with a 550 nm orange long-pass filter, the transmission spectrum of which is shown in Fig. 2.

Visible images were captured using an Epsom GT-9000 scanner and Adobe Photoshop software (version 4.0.1).

Results

An increase in latent fingerprint fluorescence was observed after electrical discharge treatment. Figure 3 shows a fingerprint deposited on silica; the left-hand side of the fingerprint was treated with the electrical discharge method and the right-hand side remained untreated. The two halves were imaged side-by-side under the same conditions.

The TLC experiments found the fingerprint residue from all donors separated into three main fluorescent bands: an orange band (Band 1), a green band (Band 2) and a green/yellow band (Band 3) in order of decreasing Rf. On close inspection Band 1 was found to occasionally split into two bands: a yellow band (Band 1b) and an orange band positioned directly below (Band 1a). This pattern of separation is consistent with results reported by Jones et al. (10) and is illustrated in Figs. 4 and 5. Fluorescent material was observed in the sample fingerprint after plate development, indicating that not all the fluorescent material was separated from the fingerprint by the TLC method. No quantitative data on fluorescent intensities were collected during the course of this study.

After electrical discharge treatment, no increase in fluorescence yield was observed in existing fluorescence bands 1, 2 and 3, but two new bands (Bands x and y) were induced to fluoresce yellow in different positions to the existing fluorescent bands (see Fig. 6). The top band (Band x) was positioned next to the solvent front overlapping with Band 1. The size and shape of Band x was not consistent with Band 1 (see Fig. 7). The new fluorescent bands were much wider, indicating a higher concentration of material. Charring of the plate revealed two brown bands that correspond in Rf and size/shape with Bands x and y (see Fig. 8). The TLC plate reacted with ninhy-

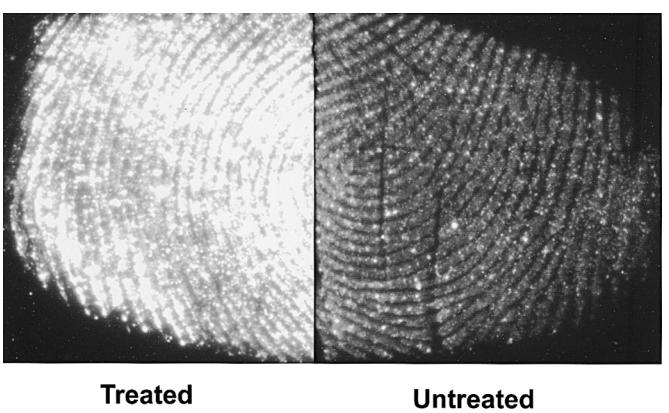

FIG. 3—*Latent fingerprint deposited on a silica gel HPTLC plate showing the increase in fluorescence observed under 514 nm laser illumination after gaseous electrical discharge treatment. One-half of the fingerprint was left untreated and the other half was treated with the electrical discharge system.*

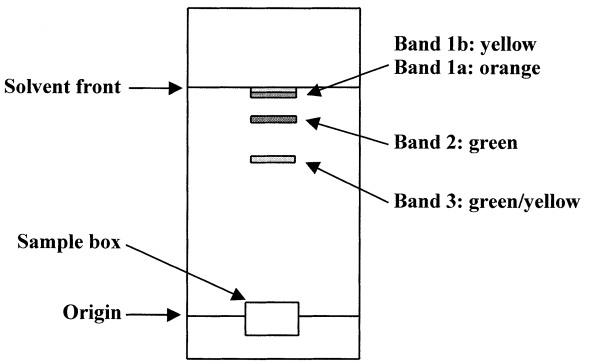

FIG. 4—*Schematic of the HPTLC separation of fingerprint residue showing the approximate position of Bands 1, 2, and 3.*

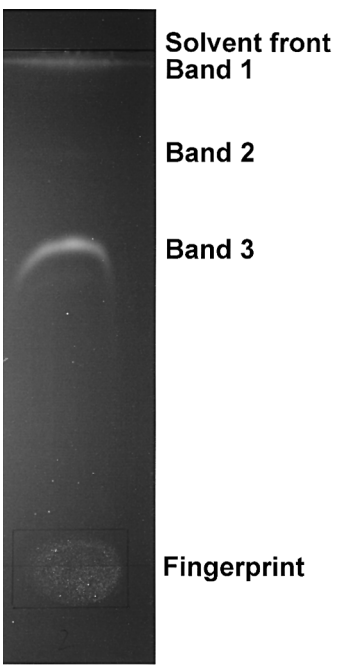

FIG. 5—*Typical separation of latent fingerprint residue on a silica gel HPTLC plate using a chloroform/methanol mobile phase showing Bands 1, 2, and 3.*

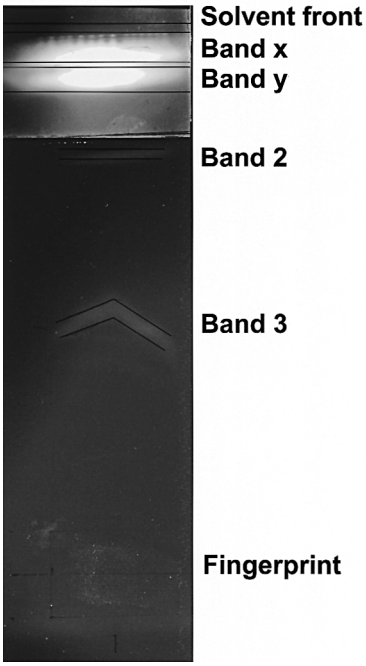

FIG. 6—*Induced Bands x and y observed after electrical discharge treatment of a HPTLC plate after separation of the fluorescent components of fingerprint residue.*

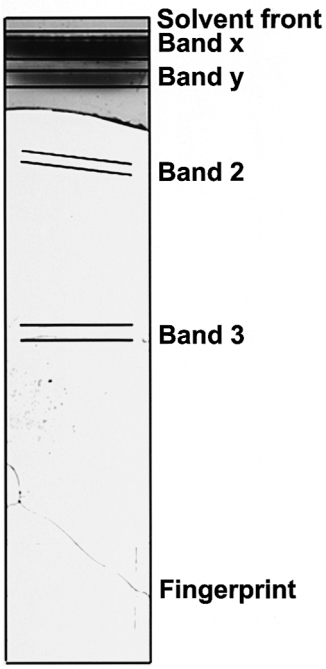

FIG. 8—*Charring of a HPTLC plate after electrical discharge treatment showing charred material in corresponding positions to Bands x and y.*

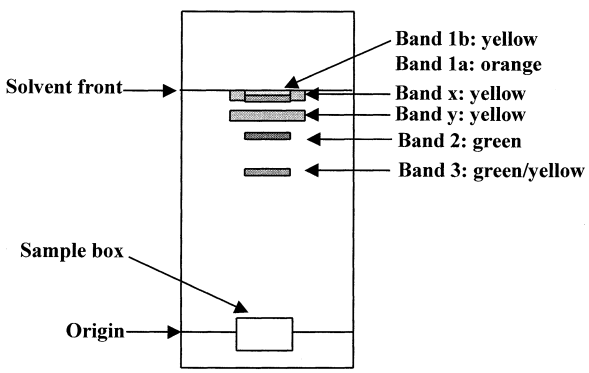

FIG. 7—*Schematic of the approximate position of HPTLC separated fluorescent components of fingerprint residue (Bands 1–3) and induced fluorescent bands after electrical discharge treatment (Bands x and y).*

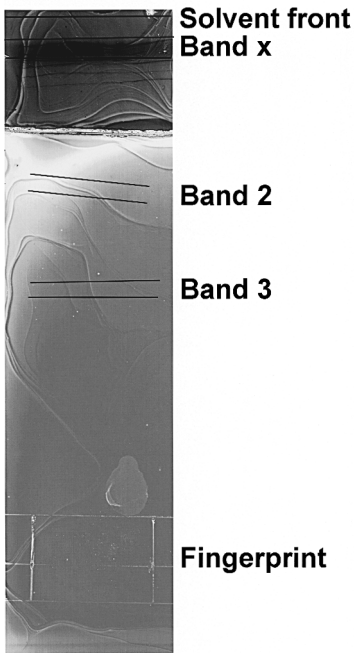

FIG. 9—*HPTLC plate after electrical discharge treatment dipped in ninhydrin solution revealing a band in a corresponding position to Band x.*

drin, resulting in a purple color across the entire plate although one and sometimes two bands deeper in color were distinguishable from the background. These bands again corresponded with Bands x and y. Occasionally Band y was not visible after ninhydrin treatment (see Fig. 9). It should be noted that none of the original fluorescent bands (Bands 1–3) charred or reacted with ninhydrin. Sometimes the ninhydrin treatment can result in "solvent marks" forming across the TLC plate as can be seen in Fig. 9. This, however, does not affect the reaction with ninhydrin in any way.

Once the location of the enhanced material had been identified, only this section was removed from the plate and treated with one or more of the visualization treatments for the illustrations.

When the fingerprint residue was separated by TLC after electrical discharge treatment, two orange/yellow bands were observed consistent with Bands x and y in the previous experiment. Likewise, treatment by charring and ninhydrin resulted in the same bands made visible as was observed previously.

Discussion

The results of this study confirm those reported by Meylan et al. (8). That is, the electrical discharge technique increases the fluo-

rescence yield from untreated fingerprint residue. This has been shown to be the case for fresh sebum-rich residue. It should be noted that no tests were carried out on eccrine-rich residue, although Meylan et al. (8) had already established that better results were achieved with sebum-rich fingerprints. The optimum treatment conditions were also found to be very similar to that of Meylan et al. (8). The pattern of natural fluorescent band separation was as found previously by Jones et al. (10).

Fingerprint residue is a highly complex mixture of chemical components, which still remain poorly characterized. The most important aspect of this work is the observation that the electrical discharge technique causes the production of new fluorophore(s) within fingerprint residue. This reaction appears to be within the lipid fraction of the residue which contains many polyunsaturated compounds including fatty acids, squalene, and wax esters (13). This is consistent with Meylan et al. (8) finding sebum-rich marks showing the greatest increase in fluorescence. These observations also correlate with previous work carried out on the use of a gaseous electrical discharge for the visualization of a variety of organic compounds (including lipids) on TLC plates (3,4). These experiments were conducted in the UV (365 nm). To confirm that the lipids also show an increase in fluorescence in the visible after treatment under the conditions described in this paper, a preliminary experiment was carried out. Eight lipid controls (wax ester, cholesterol, squalene, palmatic acid, oleic acid, monopalmatin, dipalmatin, and tristearin) were spotted onto a HPTLC plate and subjected to the electrical discharge system (as described in the experimental procedure). The results indicated that none of these lipid controls fluoresced prior to electrical discharge treatment; however, all except monopalmatin were induced to fluoresce to some extent following treatment.

The discharge may provide the initiation for lipid oxidation processes within these compounds that ultimately produce carbonyl species via peroxide intermediates. These then react with amines to produce a variety of Schiff bases. Such bases are known to fluoresce in the visible and ultraviolet (14,15). There is considerable evidence for this reaction pathway within lipid chemistry (16). It is not clear whether such reactions are occurring as a consequence of the electrical discharge technique reported here.

There is potential for this technique as a practical non–destructive method for latent fingerprint enhancement. Although more work is needed to determine the possible effects upon subsequent fingerprint enhancement methods, initial results indicate that it does not interfere with cyanoacrylate or ninhydrin enhancement processes (8).

This study shows that the electrical discharge technique is complementary to traditional methods of fluorescent detection of latent fingerprints, since the latter rely on inherent fluorescence from the non–lipid fraction (10).

Conclusions

By combining the treatment of a gaseous electrical discharge with that of illumination by 514 nm laser radiation, a new fluorophore has been observed within fingerprint residue. Preliminary TLC experiments have indicated that the fluorophore may be a derivative of lipid oxidation and subsequent reaction with nitrogenous compounds. This could provide an additional method for the location and recovery of latent fingerprints since fingerprints that do not necessarily fluoresce may do so after treatment with the gaseous electrical discharge method.

Acknowledgments

The authors would like to thank Mr. Paul Smith, Miss Charlotte Russell, Miss Clair Prance, and Mr. Ralph Thompson for their technical assistance.

References

1. Bramble SK. Fluorescence spectroscopy as an aid to imaging latent fingerprints in the ultraviolet. J Forensic Sci 1996;41(6):1038–41.
2. Segura R, Gotto AM. A new fluorimetric procedure for the detection and quantitation of organic compounds in thin-layer chromatography. J Chromatogr 1974;99:643–57.
3. Shanfield H, Hsu F, Martin AJP. Use of gaseous electrical discharge to induce fluorescence in organic compounds separated by thin-layer chromatography. J Chromatogr 1976;126:457–62.
4. Shanfield H, Lee KY, Martin AJP. Electrically activated gases as visualising agents for thin-layer chromatography. J Chromatogr 1977;142: 387–97.
5. Dalrymple BE, Duff JM, Menzel ER. Inherent fingerprint luminescence-detection by laser. J Forensic Sci 1977;22(1):106–15.
6. Duff JM, Menzel ER. Laser-assisted thin-layer chromatography and luminescence of fingerprints: an approach to fingerprint age determination. J Forensic Sci 1978;23(1):129–34.
7. Segura R, Navarro X. Use of metal salts as fluorescence-inducing reagents in thin-layer chromatography. J Chromatogr 1981;217:329–40.
8. Meylan N, Lennard CJ, Margot PA. Use of a gaseous electrical discharge to induce luminescence in latent fingerprints. Forensic Sci Int 1990; 45:73–83.
9. Creer KE. Some applications of an argon ion laser in forensic science. Forensic Sci Int 1982;20:179–90.
10. Jones NE, Davies LM, Bramble SK, Brennan JS. Separation of the visibly-excited fluorescent components in fingerprint residue by thin layer chromatography. J Forensic Sci 2000;45(6):1250–7.
11. McCaldin DJ. The chemistry of ninhydrin. Chem Rev 1960;60:39–50.
12. Friedman M, Williams LD. Stoichiometry of formation of Ruhemann's purple in the ninhydrin reaction. Bioorg Chem 1974;3:267–80.
13. Nicolaides N. Skin lipids: their biochemical uniqueness. Science 1974; 186:19–26.
14. Kikugawa K, Kato T, Hayasaka A. Formation of dityrosine and other fluorescent amino acids by reaction of amino acids with lipid hydroperoxidases. Lipids 1991;26(11):922–9.
15. Gutteridge JMC, Kerry PJ, Armstrong D. Autoxidised and lipoxidase-treated polyunsaturated fatty acids autofluorescence associated with the decomposition of lipid peroxides. Biochim Biophys Acta 1982; 711: 460–5.
16. Frankel EN. Lipid oxidation: mechanism, products and biological significance. J Am Oil Chem Soc 1984;61(12):1908–17.

Additional information and reprint requests:
Louise M. Davies, M.Sc.
The Forensic Science Service
Metropolitan Laboratory
109 Lambeth Road
London
SE1 7LP