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Use of Styryl 11 and STaR 11 for the Luminescence Enhancement of Cyanoacrylate Developed Fingermarks in the Visible and Near-Infrared Regions

Scott CHADWICK¹, Philip MAYNARD¹, Paul KIRKBRIDE², Chris LENNARD³, Xanthe SPINDLER^{1,3}, Claude ROUX¹

¹ Centre for Forensic Science, University of Technology, Sydney, PO Box 123, Broadway, NSW 2007, Australia,

² Forensic and Data Centres, Australian Federal Police, GPO Box 401, Canberra, ACT 2601, Australia,

³ National Centre for Forensic Studies, University of Canberra, Canberra, ACT 2601, Australia,

ABSTRACT

In current casework, most post-cyanoacrylate stains rely on luminescence emission in the visible region (400-700 nm). While traditional stains such as rhodamine 6G work well under most circumstances, some surfaces may generate background luminescence under the same conditions. Detection in the near infrared region (NIR >700 nm) has shown to be effective in minimising the interferences from such surfaces. The laser dye styryl 11 generated strongly luminescent fingermarks when applied after cyanoacrylate fuming on all surfaces tested. When compared to rhodamine 6G the dye was superior only when viewed in the NIR. Styryl 11 was subsequently combined with rhodamine 6G and the mixed stain formulation (named STaR 11 by the authors) induced stronger luminescence compared to styryl 11 alone with an ability to visualise in both the visible and near infrared regions. Reliable and consistent results were obtained when using either styryl 11 alone or the STaR 11 mixture. The enhancement achieved did not otherwise vary depending on the source of the fingermark

secretions. With visualisation possible in both the visible and NIR regions, the styryl 11/rhodamine 6G mixture showed significant potential as a post-cyanoacrylate stain.

KEYWORDS: Forensic science, latent fingermarks, cyanoacrylate stain, near-infrared, styryl 11, rhodamine 6G.

Current Cyanoacrylate Enhancement Stains

Cyanoacrylate fuming is one of the most effective routine techniques for developing latent fingermarks on non-porous surfaces (1). The cyanoacrylate ester selectively polymerises on fingerprint secretions to form a hard white poly-cyanoacrylate deposit. To aid in the visualisation of cyanoacrylate-developed fingermarks, luminescent stains are commonly used to increase the contrast between the substrate and the fingerprint. In order for a luminescent cyanoacrylate stain to be considered effective, it must permeate the cyanoacrylate deposit without altering or damaging it, produce sufficient luminescence under optimal visualisation conditions and produce minimal background staining. The post cyanoacrylate stains currently used in casework produce a luminescence emission at different wavelengths of the visible region (e.g. Ardrex 970-P10, Rhodamine 6G and Basic Yellow 40) (1). However, when viewing luminescent stains in the visible region, there is potential for the substrate to interfere with the luminescence emission from treated fingermarks. This is most common on brightly coloured or multicoloured surfaces or surfaces that have significant contrast (e.g. black text on a white background or a barcode). While background interferences can be reduced by using digital enhancement software, such processing can bring into question the integrity of the evidence. In some cases, a visible stain (rather than a luminescent stain) may produce better results. It would however be beneficial to have a multi-purpose luminescent stain that

could be used to enhance cyanoacrylate developed fingermarks on all surfaces regardless of background colour or pattern.

The Near Infrared Region

The infrared region ranges from 700 nm to approximately 100 μm and is divided into three sections: near, medium and far infrared. The near infrared region (NIR) ranges from 700 nm to approximately 2.5 μm . The advantage of visualising luminescent fingermarks in the NIR is that luminescence emission from the substrate at these wavelengths is highly unlikely. In the visible region many ubiquitous commercial surfaces are difficult to image in the luminescence mode due to the use of substrates or printing inks that are luminescent under the conditions typically employed. Conversely, interference of this nature is uncommon in the NIR. This suggests that fingermark visualisation in the NIR may provide a significant advantage because, without background interferences, the potential to obtain a high contrast fingermark is greatly increased.

Near Infrared Detection of Latent Fingermarks

Visualisation in the NIR has already been utilised in the biological imaging of cancer cells and immunoassays. In a forensic science context, near infrared examinations are routinely performed in the study of documents; however, very few near infrared techniques have been applied for the detection of fingermarks.

The use of near infrared filters to remove background patterns from developed fingermark samples was explored by Bleay *et al.* (2). Infrared filters have the advantage of removing the effects of inks and dyes that would otherwise interfere with a treated fingermark. This technique was only tested in conjunction with conventional latent fingermark detection techniques that included physical developer (PD), small particle reagent (SPR), vacuum

metal deposition (VMD) and powdering. This study showed the advantage of visualisation in the NIR by demonstrating the decrease in background interferences and the increase in contrast achievable at these wavelengths; however, physical developer and VMD were the only techniques that developed marks that could be visualised through infra-red filters (2). Bramble *et al.* determined that gentian violet will luminesce strongly in the NIR, significantly this means that gentian violet can be used to visualise both light and dark coloured surfaces by visible or NIR detection depending on the surface (3). Visualisation in this region would also make gentian violet a suitable technique for prints deposited on surfaces which have strong background luminescence in the visible region.

Chemical imaging in the NIR has also been used for visualisation of treated latent fingerprints (4). Chemical imaging (also known as hyperspectral imaging) is a combination of digital imaging and molecular spectroscopy that can be used for the detection of treated and untreated fingerprints in both luminescence and visible absorption modes. NIR chemical imaging has significant advantages over visible chemical imaging for fingerprint detection because of the decrease in substrate interferences (4).

While near infrared imaging methods have been evaluated for visualisation after the application of conventional development techniques, there has been very little exploration into the use of near infrared dyes for the development of fingerprints. Blackledge explored the use of carbocyanide dyes such as bis(heptamethine cyanide) for use as post cyanoacrylate stains (5). These dye proved to be useful for binding to the fatty acids present in latent fingerprints. The method described used night vision goggles with an attached CCD camera to visualise luminescence emission in the NIR (5).

Styryl Dyes as Cyanoacrylate Stains

Lennard and Mazzella explored combining the laser dye styryl 7 with other post cyanoacrylate stains (basic red 28 and basic yellow 40) to determine its effectiveness as a multipurpose cyanoacrylate stain (6). The combination was determined to have a significant Stokes shift, which resulted in broad excitation and emission wavelength ranges. However styryl 7 was found to be unstable, did not have a maximum luminescence emission in the NIR (being at 680nm) and thus still presented the some of the shortcomings as other cyanoacrylate stains that luminesce in the visible region.

The use of other styryl dyes was examined by Maynard *et al.* in a study that explored the use of styryl 8 and styryl 9M as post cyanoacrylate stains and in dye-coated nanoparticle powders (7). The dyes tested were successful in adhering to the fingerprints as well as providing sufficient luminescence emission in the NIR that was effective in reducing background interferences. The fingerprints in this study were visualised using a chemical imaging system (ChemImage CONDOR™). While there are many advantages to chemical imaging, the high instrument cost and long acquisition times are a significant drawback.

Styryl 11 (Figure 1), commercialised as a pumped-laser dye, has a maximum absorbance at 575 nm and a strong luminescence emission in the NIR (766 nm). There has been no previous published research into its use as a stain for the development of latent fingerprints.

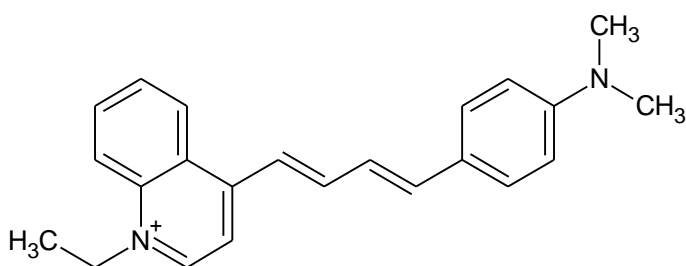


Figure 1: Styryl 11 (LDS 798) CAS No: 92479-59-9

The purpose of this study was to develop a styryl 11 based cyanoacrylate stain that would provide strong luminescence emission in the NIR. Once optimised the dye solution was to be tested on a range of substrates including surfaces that give strong luminescence emission in

the visible region. Based on the work of Lennard and Mazzella (6), styryl 11 was also combined with rhodamine 6G to determine if the mixture would extend the visualisation parameters in both the visible and near infra-red regions and essentially create a universal cyanoacrylate stain that could be used on any surface regardless of background luminescence. This dye mixture was named STaR 11 by the authors.

Materials and Methods

General

The experimental work was divided into three sections, dye optimisation, comparison study and donor study. For dye optimisation only charged fingermarks were used to ensure that sufficient cyanoacrylate was deposited. This involved donors rubbing fingers on their forehead, then rubbing their hands together for homogenisation prior to depositing fingermarks on the surface. The comparison study involved a single donor depositing a single fingermark on the surface, after fuming the fingermark was split into two and one half stained with rhodamine 6G, the other half stained with either styryl 11 or STaR 11. This was performed on all non-porous surfaces and repeated 5 times, with new solutions prepared each time. The donor study was performed with five different donors (male and female) giving both charged and natural (un-charged) fingermarks.

Substrates selected for evaluation were divided into non-porous and semi-porous surfaces (Table 1). These surfaces were chosen as they are common surfaces found in casework or they are surfaces that are typically problematic when detecting fingermarks using cyanoacrylate and conventional cyanoacrylate stains.

Table 1: Surfaces evaluated in this study

Non-porous Surfaces	Semi-porous Surfaces
Fanta® can	Coloured glossy cardboard packaging
Glass microscope slides	
Plastic bag (polyethylene)	
Zip-lock bag (polyethylene)	

Rhodamine 6G was obtained from Sigma Aldrich and styryl 11 was obtained from Lastek/Exciton. The solvents used (acetone, acetonitrile, ethanol, isopropanol, methanol and methyl ethyl ketone) were all analytical grade and were obtained through Chem Supply. The optimised working solution formulations are summarised in Table 2.

Table 2: Method to prepare 100 ml of working solution for each cyanoacrylate stain

	Styryl 11 (g)	Rhodamine 6G (g)	Acetone (ml)	Methyl Ethyl Ketone (ml)	Isopropanol (ml)	Deionised Water (ml)
Styryl 11	0.05	N/A	34	N/A	N/A	66
Rhodamine 6G	N/A	0.02	N/A	15	10	75
STaR 11	0.05	0.2	N/A	15	10	75

All samples were fumed using a Carter-Scott Design Cyanoacrylate Fuming Cabinet ‘Cyanofume FCC171’. The cyanoacrylate ester employed was Loctite ® 406™ Instant Adhesive. All samples were deposited and fumed immediately after deposition, developed samples were left for 24 hours after cyanoacrylate development before stain solutions were applied (to ensure hardening of the deposited polymer). Fingermarks were visualised using a Rofin Polilight PL500 / 500W forensic light source in conjunction with a Rofin Poliview digital image capture system (Rofin Australia Pty. Ltd.). The acquisition software was V++ Precision Digital Imaging System (version 4.0). Luminescence measurements were performed using a VSC 2000HR imaging system (Forster & Freeman, UK).

Assessment of Results

When comparing the performance of the styryl mixtures to that of the rhodamine 6G formulation employed by the Australian Federal Police (8), each fingermark was given a comparative score based on the improvement that the styryl 11 solutions had over rhodamine 6G (Table 3) (9). The amount of improvement was determined based on the strength of luminescence, ridge detail clarity and background interferences.

Table 3: Qualitative Grading System

Numerical Value	Qualitative Equivalent
-2	Significant decrease in enhancement when compared to rhodamine 6G
-1	Slight decrease in enhancement when compared to rhodamine 6G
0	No enhancement when compared to rhodamine 6G
+1	Slight increase in enhancement when compared to rhodamine 6G
+2	Significant increase in enhancement when compared to rhodamine 6G

Results and Discussion

Optimisation of Stain Formulations

Styryl 11 was soluble in all solvents tested, but the formulation that gave the best results was a 1000 ppm stock solution in acetone, diluted in a 1:2 ratio with water to use as a working solution. The stock solution could also enhance fingermarks at concentrations as low as 500 ppm (when diluted 1:2 with water) and the working solution could enhance fingermarks with

a 1:3 stock:water dilution. However, the fingerprint enhancement obtained with these solutions was no consistent.

The STaR 11 mixture extended the visualisation parameters into the visible region and also increased the luminescence emission in the NIR. The stock solution mixture that worked best was a 1:4 (styryl 11: rhodamine 6G) weight ratio subsequently diluted 1:3 with water to produce a working solution. Lower concentration ratios were tested however none of them provided a significant increase in luminescence in the NIR. For fingerprints deposited on semi-porous glossy cardboard, a 1:15 STaR 11: water working solution was used. This significantly decreased the amount of background staining produced, resulting in clearly visible fingerprints when observed in the luminescence mode.

A styryl 11 rhodamine 6G basic yellow 40 mixture was also prepared in an attempt to further extend the visualisation parameters. However this did not provide any advantage over the STaR 11 mixture and was not further investigated.

Luminescence spectra were recorded for styryl 11 and STaR 11 treated fingerprints (Figure 2 and Figure 3). Styryl 11 showed strong luminescence emission in the NIR, with the optimal excitation occurring at 590 nm and an emission maximum at 725 nm. The styryl 11 treated fingerprints on glass gave luminescence emission when viewed in the NIR (Figure 4). The STaR 11 mixture showed a significant increase in luminescence emission in the visible region (due to rhodamine 6G) as well as broader emission spectra for most excitation wavelengths. The optimal excitation wavelength for visualisation was determined to be 530 nm with an emission maximum at 683 nm. However if a higher excitation wavelength is used, such as 590 nm, then the emission maximum is shifted to 735 nm. The increase in luminescence emission intensity observed with the STaR 11 mixture was found to be due to a Förster Resonance Energy Transfer (FRET) (Figure 5) that occurred between the two dyes

when combined. The rhodamine 6G acts as a chromophore photon donor, with absorbed photons transferred non-radiatively to the styryl 11 chromophore photon acceptor that becomes excited. This results in an increase in luminescence emission when viewed under the acceptor's visualisation parameters. (Figure 6 and Figure 7)

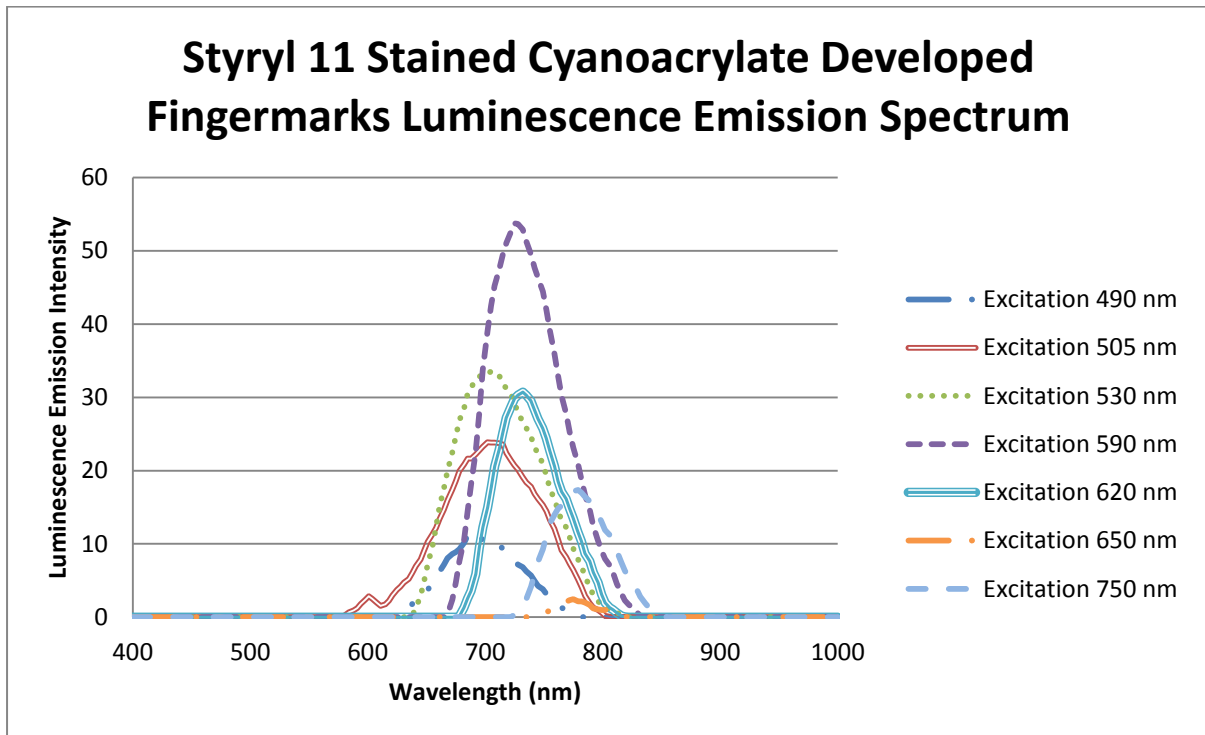


Figure 2: Luminescence spectra for a styryl 11 treated fingermark

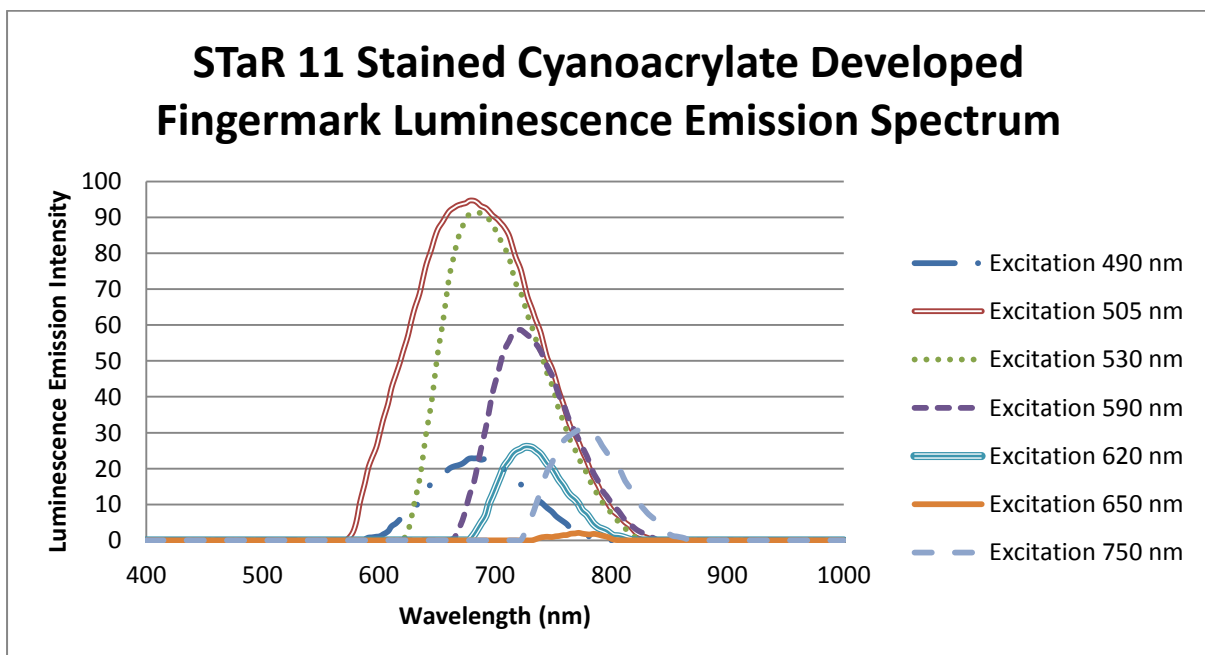


Figure 3: Luminescence spectra for a STaR 11 treated fingermark



Figure 4: Cyanoacrylate developed fingerprint on glass treated with styryl 11 and visualised in the luminescence mode (excitation 590 nm; barrier band-pass filter 750 nm)

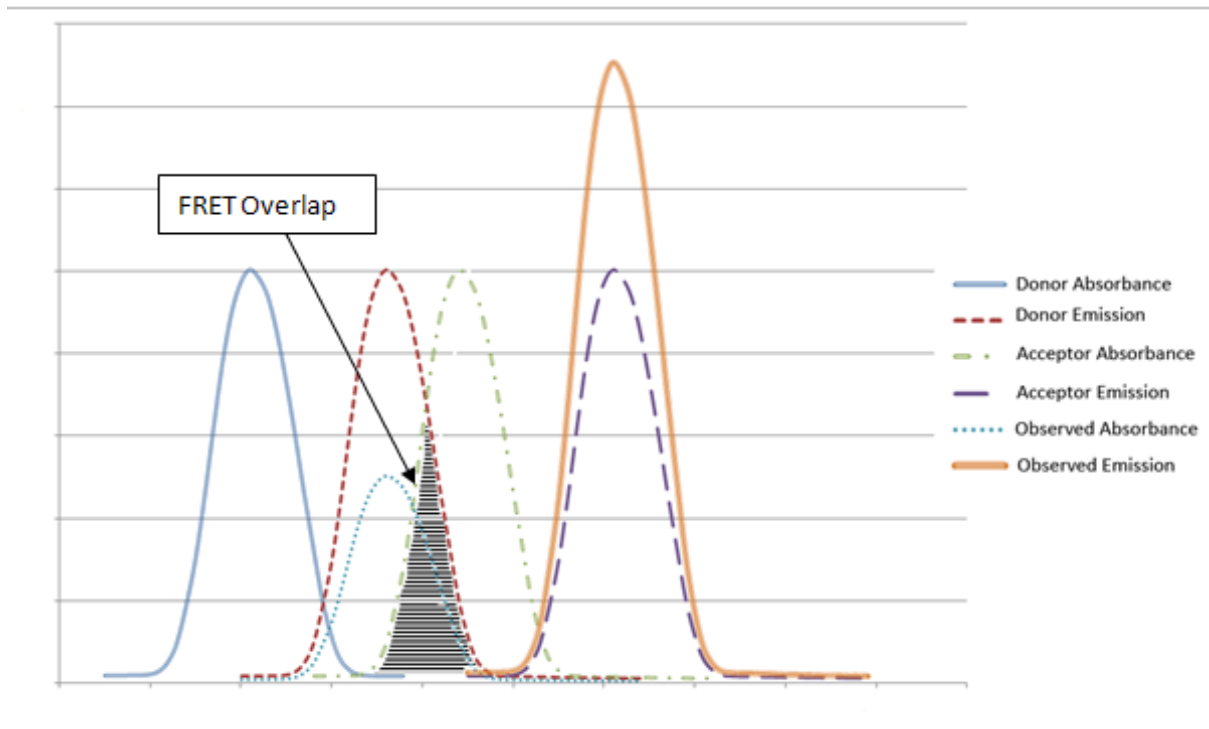


Figure 5: Förster Resonance Energy Transfer FRET Mechanism



Figure 6: Cyanoacrylate developed fingerprint on glass treated with STaR 11 and visualised in the luminescence mode (excitation 530 nm; barrier band-pass filter 610 nm)



Figure 7: Cyanoacrylate developed fingerprint on glass treated with STaR 11 and visualised in the luminescence mode (excitation 590 nm; barrier band-pass filter 750 nm)

Performance on Non-porous Surfaces

Many aluminium soft drink cans provide a highly reflective multi-coloured background that can make visualisation of treated fingermarks very difficult. When the styryl 11 treated fingermarks on such a surface were viewed in the NIR, there was a significant decrease in background luminescence (and hence background interference) compared to rhodamine 6G. However, when compared to other surfaces there was an increase in the amount of background staining from the styryl 11 solution. Despite the resulting decrease in contrast, sufficient detail was still visible for the fingermarks to be adequately visualised (Figure 8). The STaR 11 mixture provided a significant increase in luminescence that resulted in superior fingermark visualisation when compared to rhodamine 6G. The same amount of background staining was present; however, this did not prevent visualisation. While, in this case the presence of rhodamine 6G did not extend the visualisation parameters, it did increase luminescence strength (when compared to styryl 11), resulting in shorter exposure times and greater background suppression (Figure 9).



Figure 8: Cyanoacrylate developed fingermark on a Fanta® can stained with (left) rhodamine 6G (luminescence mode; excitation 505 nm, barrier band-pass filter 610 nm) and (right) styryl 11 (luminescence mode; excitation 590 nm, barrier band-pass filter 750 nm)



Figure 9: Cyanocrylate developed fingermark on a Fanta® can stained with (left) rhodamine 6G (luminescence mode; excitation 505 nm, barrier band-pass filter 610nm) and (right) STaR 11 (luminescence mode; excitation 590 nm, barrier band-pass filter 750nm)

Polyethylene bags are commonly found in routine casework and, while rhodamine 6G generally works well on this substrate, styryl 11 and the STaR 11 mixture provide suitable alternatives. For the plastic bags tested the styryl 11 stained fingermarks when viewed in the NIR region, gave strong contrast with minimal background interferences. When compared to rhodamine 6G, there was no luminescence emission in the visible region, therefore styryl 11 was superior only when viewed in the NIR region which was to be expected. Rhodamine 6G had significantly stronger luminescence emission which meant that background interferences were very low even when viewed in the visible region (Figure 10). The luminescence intensity for the styryl 11 stained marks on zip-lock bags was lower than for the styryl 11 stained marks on the substrates tested (Figure 11).

The STaR 11 mixture provided a significant improvement over the styryl 11 solution when employed on plastic and zip-lock bags as it extended the visualisation into the visible region

but also improved luminescence emission in the NIR region. When viewed at the optimal visualisation parameters, there was no difference in luminescence strength between rhodamine 6G and STaR 11 mixture (Figure 12 and Figure 13).



Figure 10: Cyanoacrylate developed fingerprint on a plastic bag stained with (left) rhodamine 6G (luminescence mode; excitation 505 nm, barrier band-pass filter 610nm) and (right) styryl 11 (luminescence mode; excitation 590 nm, barrier band-pass filter 750nm)



Figure 11: Cyanoacrylate developed fingerprint on a zip-lock bag stained with (left) rhodamine 6G (luminescence mode; excitation 505 nm, barrier band-pass filter 610nm) and (right) styryl 11 (luminescence mode; excitation 590 nm, barrier band-pass filter 750nm)



Figure 12: Cyanoacrylate developed fingerprint on a plastic bag stained with (left)

rhodamine 6G (luminescence mode; excitation 505 nm, barrier band-pass filter 610nm) and (right)

STaR 11 (luminescence mode; excitation 530 nm, barrier band-pass filter 750nm)



Figure 13: Cyanoacrylate developed fingerprint on a zip-lock bag stained with (left) rhodamine 6G (luminescence mode; excitation 505 nm, barrier band-pass filter 610nm) and (right) STaR 11 (luminescence mode; excitation 530 nm, barrier band-pass filter 750nm)

Performance on Semi-porous Surfaces

The styryl 11 and rhodamine 6G solutions bled into the glossy cardboard which resulted in a strongly fluorescent background. A more dilute styryl 11 solution was prepared and, while it did decrease the degree of background staining, it did not provide enough luminescence for the treated fingerprint to be adequately visualised (Figure 14).

The STaR 11 mixture, however, provided very promising results; when diluted (1:15 styryl:water mixture), luminescent fingerprints could be visualised but only when viewed in the NIR. The dilute solution had the advantage of decreasing background staining and reducing interferences that were present when viewed in the visible region but were not present in the NIR (Figure 15). A fingerprint deposited on a barcode on this surface was

used to test the effectiveness of the stain against a high contrast backgrounds. When the fingerprint was viewed in the visible region (Figure 16), the black lines from the barcode prevented a complete fingerprint image from being visualised. When viewed in the NIR, the treated fingerprint was not obstructed by the background and could be seen over the black lines of the barcode (Figure 17).

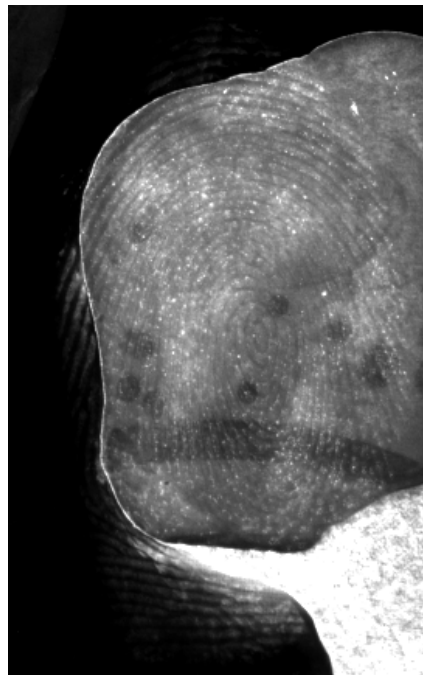


Figure 14: Cyanoacrylate developed fingerprint on glossy cardboard stained with styryl 11, (luminescence mode; excitation 590 nm, barrier band-pass filter 750nm)



**Figure 15: Cyanoacrylate developed fingerprint on glossy cardboard stained with STaR 11,
(luminescence mode; excitation 530 nm, barrier band-pass filter 750nm)**



**Figure 16: Cyanoacrylate developed fingerprint on a barcode on glossy cardboard stained with STaR 11,
(luminescence mode excitation 505 nm, barrier band-pass filter 610nm)**

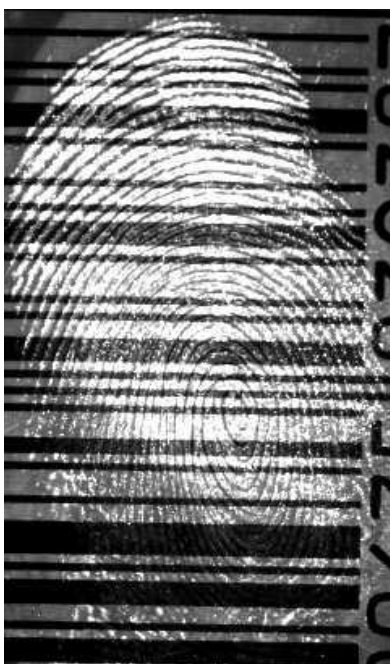


Figure 17: Cyanoacrylate developed fingerprint on a barcode on glossy cardboard stained with STaR 11, (luminescence mode excitation 530 nm, barrier band-pass filter 750nm)

Donor Study

The donor study did not show any evidence that the STaR 11 mixture was dependent upon gender or high sebaceous content. However, the results were dependent on the amount of cyanoacrylate polymer deposited, which is consistent with conventional cyanoacrylate stains. There was a noticeable difference in the amount of polymer deposition for charged and natural prints for some donors. This demonstrated that the STaR 11 mixture does not directly interact with the fingerprint secretions and will only stain fingerprints if cyanoacrylate is present. The STaR 11 mixture was able to visualise stained fingerprints on all surfaces tested. When stained on the polyethylene bags fingerprints could be visualised using either rhodamine 6G or STaR 11. Rhodamine 6G on its own was not tested on the glossy cardboard or the Fanta can because previous results had indicated that these surfaces gave strong background luminescence that resulted in poor contrast and visualisation. However, fingerprints on these substrates were stained with STaR 11(after cyanoacrylate treatment)

and luminescence imaging was performed in both the visible and near infra-red regions. In these cases, the fingermarks could not be visualised in the visible region however when viewed in the NIR, luminescence fingermarks could be observed (Figure 18 and Figure 19). These results reinforce the advantage of visualisation in the NIR region as background interferences are minimised and contrast is improved significantly. This study indicated that the STaR 11 mixture had a similar affinity for polycyanoacrylate as does rhodamine 6G, but with the added advantage of extended visualisation parameters in the luminescence mode.



Figure 18: Natural female cyanoacrylate developed fingerprint on a Fanta ® can stained with STaR 11 and visualised in the luminescence mode (right) excitation 530 nm, barrier band-pass filter 610nm; and (left) excitation 530 nm, barrier band-pass filter 750nm

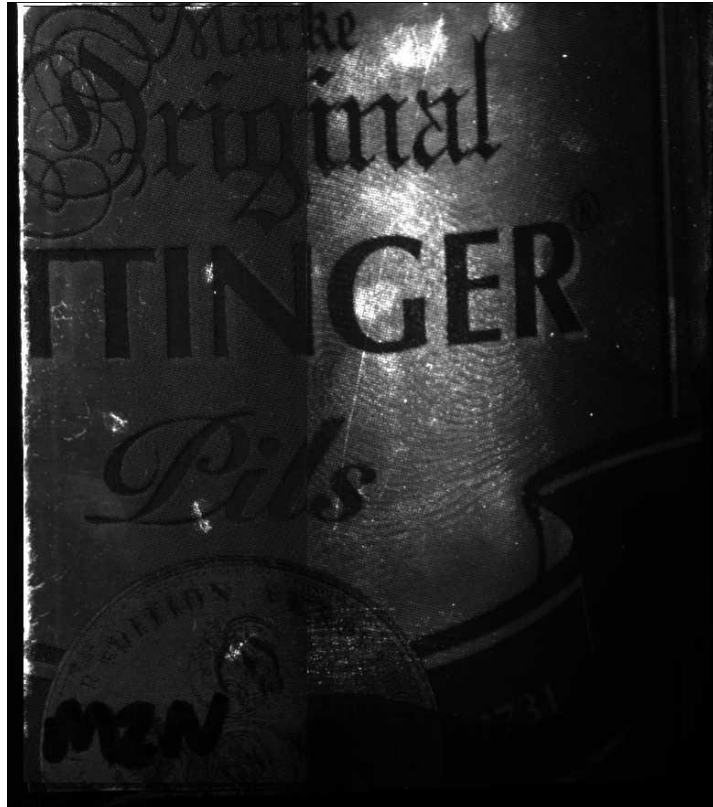


Figure 19: Natural male cyanoacrylate developed fingermark on glossy cardboard stained with STaR 11 and visualised in the luminescence mode (right) excitation 530 nm, barrier band-pass filter 610nm; and (left) excitation 530 nm, barrier band-pass filter 750nm

The donor study and the repeat experiments performed on the range of surfaces discussed above indicated that styryl 11, on its own, or mixed with rhodamine 6G is a robust and universal cyanoacrylate stain that gives repeatable results. Figure 20 and 21 indicate that when compared to rhodamine 6G, the styryl 11 dye formulations give superior results in the NIR. However, the STaR 11 mixture provides greater consistency when compared to styryl 11, and a broader visualisation range when compared to rhodamine 6G and styryl 11 on their own.

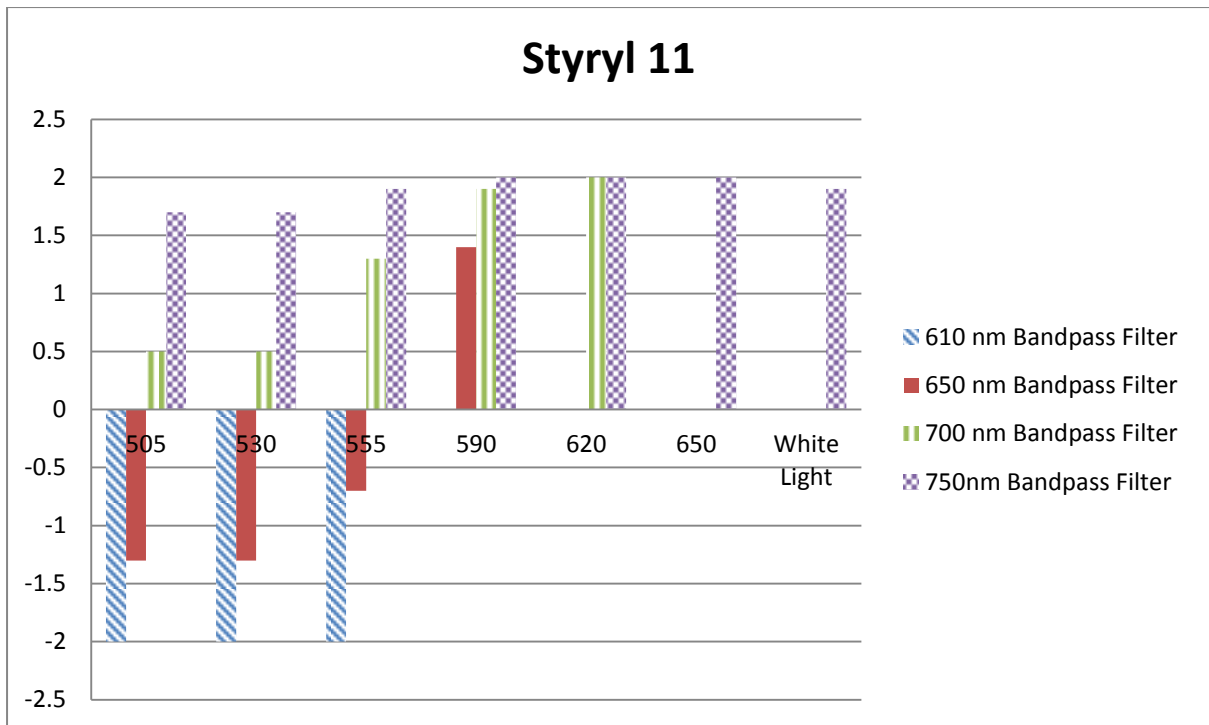


Figure 20: Average comparison values for styryl 11 versus rhodamine 6G on all non-porous surfaces tested (A negative value indicates better rhodamine 6G performance, a positive value indicates better styryl 11 performance)

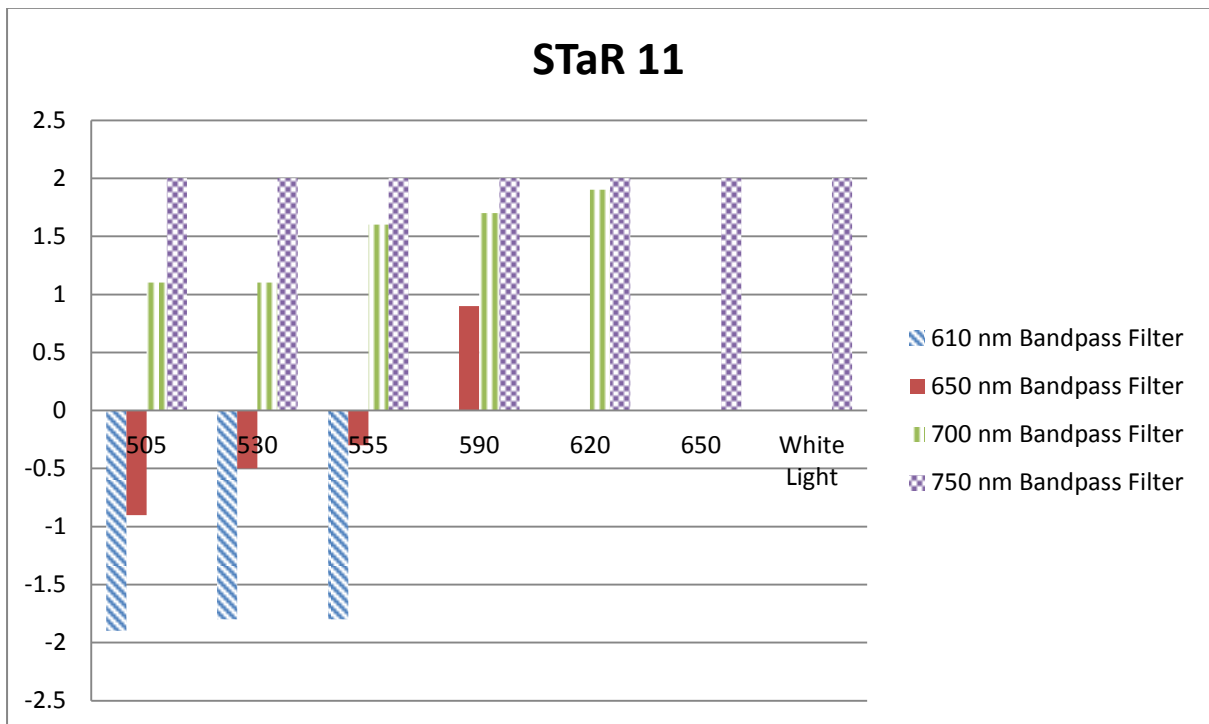


Figure 21: Average comparison values for STaR 11 versus rhodamine 6G on all non-porous surfaces tested (A negative value indicates better rhodamine 6G performance, a positive value indicates better STaR 11 performance)

Conclusions

This study evaluated the use of the laser dye styryl 11 as a post cyanoacrylate stain, comparing its effectiveness to rhodamine 6G on its own and to mixtures of styryl 11 and rhodamine 6G. Fingermarks were deposited on a range of different surfaces that were selected based on their frequency of occurrence in casework or because of surface interferences when viewed in the visible region. Styryl 11 was found to be soluble in all polar solvents tested and could be visualised on all surfaces tested except for glossy cardboard. Rhodamine 6G displayed strong luminescence emission on the polyethylene bags; however, on the other surfaces tested rhodamine 6G was unsuitable. Styryl 11 was only superior to rhodamine 6G when viewed in the NIR, which increased contrast but longer exposure times were required in order to visualise in this region. Even when viewed in the NIR, there was a lack of consistency with the styryl 11 staining process and it will not always provide acceptable results compared to what may be achieved using rhodamine 6G.

However, when styryl 11 was combined with rhodamine 6G, the mixed stain formulation (STaR 11) provided a significant improvement over each individually in both luminescence emission intensity and visualisation parameters (ie broad excitation and emission characteristics). The ability to visualise in both the NIR and visible region while using only a single reagent offers significant advantages. The Förster Resonance Energy Transfer (FRET) mechanism that occurs between the two dyes also assists in improving the luminescence

emission in the NIR. This also meant that background interferences were kept to a minimum, thereby significantly improving the overall contrast. The most significant application of the STaR 11 mixture was its use on cyanoacrylate-developed fingermarks developed on multicoloured glossy cardboard. When viewed in the visible region the surface colour prevented any luminescence being observed from rhodamine 6G treated fingermarks. However, when treated with STaR 11 and viewed in the NIR, there was a dramatic improvement resulting in a clear fingermark being visualised. The only drawback of this technique on this surface was the presence of background staining that, in some cases, decreased overall contrast, however, this did not prevent visualisation. High contrast surfaces (such as barcodes) were also suppressed because, when visualised in the NIR, the background interferences were suppressed enough to enable visualisation on both black and white sections of the surface.

The donor study using male, female, natural and charged fingermarks emphasised the universal nature and the lack of donor dependence of the STaR 11 dye mixture.

Based on these results, visualisation in the NIR has been shown to result in increased fingermark contrast due to the suppression of background luminescence as well as eliminating interferences from the surface colour. The use of styryl 11 in conjunction with rhodamine 6G provides a novel alternative to conventional cyanoacrylate stains with the added advantage of being able to visualise treated fingermarks in both the visible and the NIR.

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Addition information and reprint requests:

Philip Maynard, Ph.D.

Department of Chemistry and Forensic Science,

University of Technology Sydney

PO Box 123

Broadway

NSW 2007

Australia

Email: Philip.Maynard@uts.edu.au