Forensic Light Sources for Detection of Biological Evidences in Crime Scene Investigation: A Review

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ABSTRACT: Identification of biological evidences, such as blood, semen, saliva and urine, are important for crime scene investigation. Forensic light sources have been used for detection of biological evidences, where this method is a simple, presumptive, non-destructive test and applicable for detecting most types of biological evidences. Biological evidences can be detected by forensic light source due to their natural characteristic, such as light absorption (blood) or fluorescence effect (semen, saliva and urine). Biological evidences on different materials would have different effect in detection, where materials with high absorbent or exhibit strong fluorescence would affect the detection of biological evidences. This paper reviews on the methods and limitation of detecting biological evidences by forensic light source and provide the recommendations for improving the detection techniques using forensic light source.

Keywords: Forensic light source, biological evidence, blood, semen, urine, saliva

Introduction

Biological evidences such as blood, semen, saliva and urine are among the most important evidences in crime scene investigation [1]. Valuable information can be obtained from the biological evidences found, such as DNA evidence for the identification of the victims and suspects and the bloodstain pattern for the determination of the sequence of events [1]. Several methods have been developed for the identification of these biological evidences, which can be divided into presumptive tests and confirmatory test. Presumptive tests are just screening tests, whereas confirmatory tests will conclusively identify the species of the particular evidence [1]. However, most of the tests are destructive test, where the DNA evidence would be destroyed, and some tests can only be carried out in a laboratory [1]. One of the simplest presumptive tests that can be used to determine most of the biological evidence is forensic light source (FLS) [1].

FLS is a term used commonly to refer to an illumination system adapted in forensic application, such as laser and high-intensity filtered lamps [2]. A non-laser FLS is sometimes referred as an alternative light source (ALS) [2]. FLS can either make the evidence fluorescence or enhance the contrast of the evidence against the background [3]. Fluorescence happens when the FLS emitted to the biological evidences, such as semen, saliva and urine, where these fluids absorbed light at particular wavelength and then re-emits the absorbed energy as light at a longer wavelength [2]. Besides, FLS can be used to enhance contrast of bloodstain on dark surfaces, where the stain is not visible to naked eye, such as bloodstain [3].

The maximum detectable dilution of biological stains by FLS was relatively lower compared to chemical based method, such as luminol for bloodstains, where maximum detectable dilution of bloodstains was 1/1000 using Polilight® [4], whereas luminol sensitivity was up to 1/5000000 [5]. However, since no chemical was required and it was easy to use, FLS was commonly used in crime scene investigation as a scanning tool. Besides, most of the FLS was used to enhance the biological stains towards its background for photography purposes. Moreover, FLS was suitable in detecting various types of biological evidence [1].

This paper reviews the methods of detection using FLS, brief overview of different types of available FLS and also the effects of biological evidences on different surfaces towards the detections. This paper is organized as follows:

- Introduction of the responses of biological evidences, such as blood, semen, saliva and urine towards different light wavelength of FLS.
- Discussion of the detection of biological evidences using different types of FLS. The comparisons between each FLS that have been reported in literature in discussed in this section.
- Summary of the effects of biological evidences on different surfaces towards their detections using FLS. This section reported the maximum

detectable dilution of biological evidence on different surfaces using FLS that were found in literature.

Responses of Biological Evidences towards FLS

(a) Blood

Untreated dry blood does not show a significant fluorescence effect but it has a high absorption in a very broad region of light wavelength from 300-900nm, which cover the entire light wavelength, including UV, visible (VIS) and IR light [6]. Hence, bloodstain will occur as a dark spot when it was exposed to any type of light. Most of the FLS were able to enhance the contrast of bloodstain towards its background, especially on dark background [1-13]. Bloodstain would appear to be brighter against a dark background [2].

Along the high absorption region, the strongest absorption band occurred in a narrow band of 395nm to 435nm, with the maximum absorption at 415nm due to the presence of haemoglobin, as shown in **Fig. 1** [6]. Background correction method was proposed to further enhance the dry untreated blood towards the background using this narrow wavelength of light [11,12]. In their experiment, the light source used was Rofin PL-10 Polilight, which is a high intensity xenon lamp with selectable narrow bandpass filters. These bandpass filters can be adjusted to emit the light with peak at 435nm, 415nm and 395nm. Due to the difference in absorption between 395nm, 415nm and 435nm light, this method can enhance the bloodstain images that are unclear even at 415nm, where the background exhibits almost the same absorption of light [11,12].

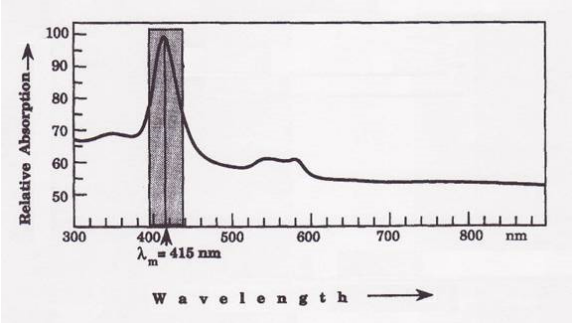

Fig. 1: Absorption Spectrum of Dry Blood [6]

(b) Semen

Untreated dry semen is a very strong photoluminescence substance [6], where it would absorb certain wavelength of light, excitation spectrum, and re-emit a longer wavelength of light, emission spectrum [2,4]. Stoilovic [6] reported that the emission spectrum of semen stain was covering the region of 400nm-700nm with the excitation spectrum measured in 300-480nm by Polilight®, as shown in **Fig. 2**.

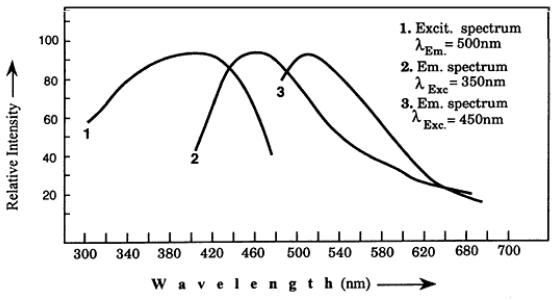

Fig. 2: Photoluminescence spectra of dry untreated semen [6]

By supplying the specific excitation light with appropriate goggles or filters, semen stain can be clearly observed due to the photoluminescence effect of semen. Goggles were used to filter out strong excitation wavelength and only allowed the emission wavelength to pass through [6]. **Table 1** summarized the procedures for detection of untreated semen stain using different types of excitation light with the appropriate goggles or filter, which was reported by Stoilovic [6]. The test was run from the combination of UV light without goggles to the combination of green-yellow light with violet filter. Suitable light with goggles combination was chosen when the background was not photoluminescence during observation [6].

In a more recent paper reported by Nelson and Santucci [14], a test to determine the best combination exciting wavelength and goggles for viewing semen stains was done using Omniprint 1000, an adjustable wavelength light source with narrow band increments (30-40nm) between 320nm to 510nm and different coloured goggles, which were yellow, orange and red goggles. The best fluorescence effect of semen stains was found at the wavelength of 420nm and 450nm, observed with human eyes through orange goggles. It was also reported that semen stains would appear as a yellow-greenish stains when exposed to a continuous green beam at 532nm wavelength. These continuous green beam was generated from a laser's type FLS, Spectra-Physics® Reveal™, and the semen stains observed through a orange laser safety goggles that block 532nm wavelength [9].

Table 1: Excitation light with appropriate goggles for untreated semen stains detection recommended by Stoilovic [6]

Excitation light	Goggles/Filters	Colour of the observed stain
UV	No goggles needed, but recommended to wear UV safety goggles	Blue
Violet	Yellow goggles	Yellow
Blue	Yellow goggles	Yellow
Green	Orange goggles	Orange
Green-yellow	Red goggles	Red
Green-yellow	Violet filters (425nm)	Black

(c) Saliva

Dried saliva stain is virtually colourless and difficult to detect by naked eye [15]. From literature, saliva stain exhibits fluorescence effect but in a lower intensity compared to semen [15]. Saliva stain was detectable by naked eye when exposed to UV light [9], where it would appear bluish-white, but this would not differentiate it from other stains [1,16]. In addition, UV-UV photoluminescence, where excitation wavelength at short UV (266nm) and emission wavelength at long UV, was reported to be able to detect saliva stain [10]. Besides, saliva stain was also detectable under excitation wavelength of 450nm with orange goggles [4] or 555nm interference filters, which is a filter that use interference effect to transmit 555nm wavelength of light and reflect other wavelengths [15]. Camilleri *et al*. [15] reported that the optimum contrast of saliva stains on white cotton background was achieved using the 470nm excitation wavelength with the 555nm interference filters, while saliva stain was also detectable with human eyes using other excitation wavelength with different colour goggles or filters, such as 415nm with yellow goggle or 555nm interference filters, 470nm with 530nm interference filters, 490nm with 555nm interference filters and 505nm with 555nm interference filters. Furthermore, saliva stain appeared to be white thin edged stain when exposed to 450nm excitation wavelength and viewed with orange goggles [4]. Besides, yellow-orange stain was observed when saliva stain exposed to 532nm excitation wavelength and viewed with goggles that designed to block 532nm light [9]. **Table 2** summarized the excitation wavelength with the suitable goggles or interference filters for detection of saliva by human eyes.

Table 2: Excitation wavelength with suitable goggles or filters for detection of saliva

Excitation light	Goggles/Filters	Colour of the observed stain
Long UV [1,9,16]	No goggles needed, but recommended to wear UV safety goggles	White-bluish
415nm [4,15]	Yellow goggles/555nm interference filters	Not stated in literature
450nm [4,15]	Orange goggles/555nm interference filters	White (Orange goggles)
470nm [15]	530nm/555nm interference filters	Not stated in literature
490nm [15]	555nm interference filters	Not stated in literature
505nm [15]	555nm interference filters	Not stated in literature
532nm [9]	Goggles that block 532nm light	Yellow-orange

Notes: interference filters allow only the desirable wavelength pass through.

(d) Urine

Urine stains are hard to be seen because the nature of urine, where these stains will become diluted on fabric surfaces [1]. In fact, urine stains exhibits fluorescence effect when exposed to UV light, but the colour of the stain may vary in the presence of abnormal substances, such as glycosuria [16]. Vandenberg and Oorschot [4] reported that urine was detectable by human eyes under 415nm excitation wavelength with yellow goggles, 450nm excitation wavelength with orange goggles and 505nm excitation wavelength with red goggles. Besides, Seidl *et al*. [9] tested urine stains with excitation wavelength at 532nm using Spectra-Physics® Reveal™. From the results of their test, when urine stains were viewed under goggles that block 532nm light, the stain appeared as a yellow-orange stain but more intense compared to saliva, which was also exhibits the same colour under this wavelength/filters combination. **Table 3** summarized the excitation wavelength with the suitable goggles or filters for detection of urine.

Table 3: Excitation wavelength with suitable goggles or filters for detection of urine

Excitation light	Goggles/Filters	Colour of the observed stain
UV [16]	No goggles needed, but recommended to wear UV safety goggles	Depends on abnormal substance presence
415nm [4]	Yellow goggles	Not stated in literature
450nm [4]	Orange goggles	White
505nm [4]	Red goggles	Not stated in literature
532nm [9]	Goggles that block 532nm light	Yellow-orange

Detection of Biological Evidences using Different Type of FLS

There were several FLS reported for the success in aiding the detection biological evidences by human eyes. In order to increase the sensitivity of detection, an FLS must produce a high intensity of light, as Wawryk and Odell [13] reported that most FLS with lower intensity was not suitable to be used to detect urine due to its weak emission light. Besides, different substances have different excitation wavelengths that would give the best detection. Due to these two circumstances, among all the available FLS, the most tested FLS in literature was Polilight®, where this FLS gives variety of wavelength with high intensity of light. Vandenberg and Oorschot [4] reported the test of the latest Polilight®'s model, PL500, on blood, semen, urine and saliva and it shows positive detection for all type of tested stains when observed through suitable goggles.

In fact, lasers, such as TracER and Spectra-Physics® Reveal™, have higher intensity and a narrower bandwidth compared to most of the FLS, which make it to be more effective in detection compared to other light source [3]. In an earlier paper, Auvdel [17] reported that a laser, Spectra-Physic Model 171-19, was more effective in detecting semen, saliva and sweat stains compared to Mineralight, short UV light source with 245nm. However, in another paper reported by Auvdel [18], high-intensity quartz arc tube, Luma-Print, has a better detection rate of semen, saliva and sweat compared to laser, Spectra-Physic Model 171-19. Furthermore, Luma-Print has a better portability over high-powered laser. In addition, lasers are more costly and heavier compared to other FLS [3].

The comparisons of the detection blood, semen, saliva and urine between Lumatec Superlite 400, a tunable wavelengths FLS with several different goggles, and Spectra-Physics® Reveal™, with goggles that block 532nm light emitted from the laser, were reported by Seidl *et al*. [9]. Both light sources showed comparable results in detection but fluorescence of urine was stronger with laser system. However, blood stain cannot be viewed using the laser system of 532nm wavelength with a safety goggles that block 532nm light [9]. The contrast between blood stains with the background cannot be enhanced as blood stains absorbed completely the light from laser and the light reflected from the background was blocked by the safety goggles.

Due to the strong absorption wavelength of blood stain in the entire light wavelength, there were several types of suitable FLS for bloodstains' detection reported in literature, such as high intensity LED [13], UV [8,10], Lumatec Superlite 400 [9], Poliray™ [13] and Polilight® (1-2; 4-6; 11-12). Besides, IR light was also proven to be successful in detecting bloodstain on black fabrics [7]. Moreover, it was reported that bloodstain that was covered by paint can also be revealed using Polilight® [4].

Santucci *et al*. [19] reported that Wood's Lamp, an ultraviolet light source that emits wavelengths of approximately 320-400nm, was unable to be used to distinguish between semen and other substances that were commonly found on perineum of children or adolescents. Moreover, all the 29 semen samples used for the study did not show any fluorescence when exposed to Wood's Lamp. Bluemaxx BM500, a FLS with a broad-band wavelength of 390-500nm was tested and reported to have a better performance compared to Wood's Lamp [14]. Using Bluemaxx BM500, semen stain on a white 100% cotton surface can be detected all of the time and after a brief training session, 15 out of 18 of the physicians, about 83.3%, were able to differentiate semen stain from other common product. With this FLS, the semen sample would still exhibit fluorescence with the same intensity after a few months from the initial placement on the cloth [14]. Poliray™ with 550nm camera filter was also tested to be successful in detecting semen stains by Lincoln *et al*. [20].

The detection of blood, semen, saliva and urine by different type of LED, ranging from 370 to 480nm, and Poliray™ on skin was tested by Wawryk and Odell [13]. Semen and blood stains were successfully detected by all of the tested FLS. However, a very close distance between those FLS to the surface was needed, which was less than 3cm. In addition, those stains can only be observed with filter goggles in a distance around 20cm from the

stains. Besides, saliva was not fluorescence under any type of the tested light source. This shows those tested FLS were having lower light intensity output compared to Polilight®, which caused saliva undetectable, as saliva shows weak fluorescence under Polilight®. Besides, urine was not detected by any lower intensity LED or everLED™ Maglite™ replacement bulb, but some fluorescence observed when urine exposed to Luxeon™ Star V LEDs and Poliray® [13].

There was another test by Carter-Snell and Soltys [21] to compare different wavelength effect on biological stain, such as semen, urine and saliva, using Mineralight, Evident Products CE, long UV light with 365nm, Bluemaxx BM500 and Bluemaxx Mini, blue light with 450nm. Semen stains were detectable by all the tested FLS good results, while urine was only detectable using Mineralight and Bluemaxx BM500, with weak sensitivity. Saliva stains were also fluorescence when exposed to each of the tested FLS except for Bluemaxx Mini, due to its lower intensity of light. However, the fluorescence of the same semen stain was observed to be different in colour by different examiner with the same FLS and goggles. Blue, blue-white and light green fluorescence colours of semen stain were observed when exposed to Evident Product CE, whereas white, yellow-white and green fluorescence colours were observed when exposed to Bluemaxx BM500 and Bluemaxx Mini with orange goggles.

Table 4 summarizes the wavelength output and the detectable stains reported for all of the FLS found in recent literature, such as TracER [3], Spectra-Physics® Reveal™ [9], Poliray™ [13,20], Polilight® PL500 [4], Lumatec Superlite 400 [9], Wood's lamp [19], Bluemaxx BM500 [14,21], Bluemaxx Mini [21], Evident Product CE [21], Mineralight® [21] and high intensity LED [13].

Table 4: Comparison of the tested FLS in recent literature in term of wavelength and detectable stains reported

FLS	Wavelength (nm)	Detectable Stains Reported
TracER (Laser) [3]	532 (Green laser beam)	Semen, Saliva, Urine
Spectra-Physics® Reveal™ (Laser) [9]	532 (Green laser beam)	Semen, Saliva, Urine
Poliray™ [13,20]	415-610 (mounting interference filters)	Blood, Semen, Urine
Polilight® PL500 [4]	Adjustable from UV, 415-650nm and white light	Blood, Semen, Saliva, Urine
Lumatec Superlite 400 [9]	Adjustable from 320-700	Blood, Semen, Saliva, Urine
Wood's lamp [19]	320-400 (Long UV)	Semen (doubtful)
Bluemaxx BM500 [14,21]	450 (Blue)	Semen, Saliva, Urine
Bluemaxx Mini [21]	450 (Blue)	Semen
Evident Product CE [21]	365 (Long UV)	Semen, Saliva, Urine
Mineralight® [21]	254 (Short UV)	Semen, Saliva, Urine
High Intensity LED [13]	Variety of wavelength depends on the LED used	Blood, Semen (Urine was detectable by Luxeon™ Star V LED)

Effect of Biological Evidences on Different Surfaces towards their Detection using FLS

The sensitivity of biological stains detection through human eyes using FLS varies on different type of surfaces the stains occurred. This was due to different reactions of different type of surfaces' materials towards FLS. Some materials were dark in colour, highly absorbent of liquids or exhibits strong fluorescence effect when exposed to FLS [22]. High absorbent material would absorb the biological evidences into its fabric before it dry and fluorescence of the background would mask the fluorescence of the biological stains. These factors will reduce the contrast enhancement of biological stain towards background [4]. **Table 5** shows the surfaces that had been tested in literature for the detection of biological evidences using FLS.

(a) Blood

The detection of bloodstain was poor on highly absorbent polar fleece [4]. The maximum detectable blood dilution reported was 1/1000, where the stain was on white cotton and FLS used was Polilight® PL500 [4]. However, the same stains was detectable using natural light, which means FLS has little benefit for detection of bloodstains on light-coloured surfaces [4].

Wagner and Miskelly [11,12] reported their background correction method was able to detect blood dilution up to 1000-1600, with the aid of Polilight®. They reported that using Polilight® at 415nm, the maximum detectable dilution on white cotton was 1/400, but the detectable dilution was further improved by their proposed method.

Besides Polilight®, Lumatec Superlite 400, adjusted to output the light with wavelength of 415nm, was also tested on different type of materials by Seidl *et al*. [9] and its maximum detectable dilutions were shown in Table 6. Meanwhile, Wawryk and Odell [13] reported that the bloodstain on skin was detectable using high intensity LED or Poliray™, but not visible on the second day of the experiment. From literature, IR light was used to detect bloodstain on black colour surfaces, where the stains were barely visible [7].

The maximum dilutions of bloodstains on different materials detected by the stated FLS were summarized in **Table 6**.

Table 5: Surfaces tested for detection of biological evidences with FLS in literature

Item	Surface	Colour of surfaces
1	Fabric: Cotton [9]	–
2	Fabric: Cotton (4; 11-12; 21)	white
3	Fabric: Cotton [4]	red
4	Fabric: Cotton [4]	pink with white polka dot
5	Fabric: Cotton [4]	checked weave
6	Fabric: Cotton [4,7]	black
7	Fabric: Cotton [4]	yellow, green, pink, blue, brown, purple
8	Fabric: Wool [4]	white, yellow, green, red, blue
9	Fabric: Nylon [4]	white, blue
10	Fabric: Nylon [4]	pink
11	Fabric: Polyester [4]	white
12	Fabric: Velour [4]	blue
13	Fabric: Satin [4]	pink
14	Fabric: Crepe [4]	black
15	Fabric: Polyester + spandex [4]	white, black
16	Fabric: Cotton + elastane [4]	blue
17	Fabric: Nylon + elastane [4]	white
18	Fabric: Polysester + cotton [4]	black + white
19	Fabric: Polar fleece [4]	green
20	Synthetic carpet [4]	white, blue
21	Pine wood [4]	–
22	Dried leaves [4]	–
23	Glass [4]	–
24	Brick [4]	–
25	Metal [4,9]	–
26	Plasterboard [4]	–
27	Condom [4]	–
28	Tile [9]	–
29	Glass [9]	–
30	PVC [9]	–
31	Formica [9]	–
32	Carpet [9]	–
33	Stone [9]	–
34	Wood [9]	black
35	Fabric: 35% rayon & 65% polyster [7]	black
36	Fabric: 35% cotton & 65% polyster [7]	black
37	Fabric: 35% polyster & 65% cotton [7]	black
38	Fabric: 100% velvet [7]	black
39	Fabric: 50% acrylic & 50% wool [7]	black
40	Fabric: 5% lycra & 95% cotton [7]	black
41	Fabric: 5% spandex & 95% polyester [7]	black
42	Fabric: 30% polyster &70 % rayon [7]	black
43	Fabric: 30% acrylic &70% wool [7]	–
44	Human skin [13,21]	–

Remark: " – " not found in literature

Table 6: Detectable bloodstains on different surfaces reported using FLS

FLS	Wavelength (nm) / Detection methods	Surfaces	Maximum visible dilution reported
Polilight®	Background Correction [11,12]	2	1/1600
	415nm [4]	2	1/1000
		3-18,20-26	–
		19	1
Lumatec Superlite 400	415nm [9]	1	**1/10**
		28	**1/10**
		29	**1/10**
		30	**1/100**
		31	**1/10**
		32	**1/1**
		25	**1/1**
		33	**1/1**
		34	**1/100**
High Intensity LED	370-480nm [13]	44	*nv2*
Poliray™	450nm [13]	44	*nv2*
IR	>930nm [7]	6	**1/4**
		35	1
		36	**1/4**
		37	**1/4**
		38	**1/4**
		39	**1/4**
		40	1
		41	**1/4**
		42	**1/8**
		43	**1/4**

Code: Surfaces numbers referred to the surfaces numbered in Table 5
Bold dilutions mean strong or clear detection.
Non-bold dilutions mean weak detection.
"–" indicates not stated in literature
nv2 means neat stains visible on first day but not visible on second day

(b) Semen

Vandenberg and Oorschot [4] reported that the most useful wavelength and goggles used for detection of semen stain was 450nm with orange goggles. Semen stains can be detected using FLS on most of the surfaces, where the stains was not detectable by naked eye under natural light, due to the strong fluorescence effect of semen stains [4].

Absorbency of fabric was not affecting much the detection of semen stains, where semen stains on highly absorbent surfaces, such as blue velour and dark green polar fleece, were easily detected by Polilight® [4]. The reported maximum detectable dilution on white cotton using Polilight® was 1/100 and not detectable under natural light [4]. However, the colour and pattern of the surfaces was found to affect the appearance of semen stains. The reported surfaces were pink nylon, red cotton, pink cotton white polka dot and checked fabrics, where semen stains on those same materials with different colour show good results in detection [4].

Fabrics such as white cotton, pink satin and pink fleecy material, which shows strong fluorescence under certain wavelength, reduced the contrast between semen stains and the background [22]. White cotton's excitation wavelength was about 340-410nm with 440-470nm emission wavelength, while pink satin's excitation wavelength was around 490-530nm with 570-620nm emission wavelength. Hence, the excitation wavelength with 450nm is better than UV light for detection of semen stain on white cotton [22]. Moreover, as reported earlier, Santucci *et al*. [19] tested that Wood's lamp, a long UV light source with the wavelength about 320-400nm, could not be used to detect semen stains on white or black cotton. Another FLS, Bluemaxx BM500, a FLS with the wavelength of 450nm, showed good results in detecting semen stains on white cotton [14].

The detection of semen on different surfaces between Lumatec Superlite 400 with Spectra-Physics Reveal laser was comparable, where the detectable dilution between both FLS was the same on every tested surface [9]. Wawryk and Odell [13] reported that semen stains on skin did not show any fluorescence when exposed to low intensity LED, where different LEDs' wavelengths ranging from 375nm-480nm were tested. When the same stains on skin exposed to Poliray™ and high intensity LED, such as Luxeon™ LED, fluorescence was observed when viewed with orange goggles. However, the fluorescence was very faint when

exposed to the same FLS on the second day. Besides, four FLS, Mineralight, Evident Products CE, Bluemaxx BM500 and Bluemaxx Mini, were tested and successful in detecting semen stains on human's arm [21].

The maximum dilutions of semen stains on different materials detected by the stated FLS were summarized in **Table 7**.

Table 7: Detectable semen stains on different surfaces reported using FLS

FLS	Wavelength (nm) / Detection methods	Surfaces	Maximum visible dilution reported
Polilight®	UV light [22]	2	1
	450nm + Orange goggles [4]	2,11	1/100
		3-5,10	1
		6-9,12-27	–
Bluemaxx BM500	450nm + Orange goggles [14,21]	2	–
		44	–
Mineralight	254nm [21]	44	–
Evident Products CE	365nm [21]	44	–
Bluemaxx Mini	450nm [21]	44	–
Lumatec Superlite 400	415nm + Orange goggles [9]	1	**1**
		28	1/10
		29	**1/10**
		30	**1/10**
		31	1/100
		32	**1/10**
		25	**1/10**
		33	**1**
		34	**1**
Spectra-Physics Reveal laser	532nm + 532nm block goggles [9]	1	**1**
		28	1/10
		29	**1/10**
		30	**1/10**
		31	1/100
		32	**1/10**
		25	**1/10**
		33	**1**
		34	**1**
High Intensity LED (Luxeon™ LED)	452.9nm/466.9nm + orange goggles [13]	44	*nf2*
Poliray®	450nm + orange goggles [13]	44	*nf2*

Code: Surfaces numbers referred to the surfaces numbered in Table 5
Bold dilutions mean strong or clear detection.
Non-bold dilutions mean weak detection.
"–" mean not stated in literature
*nf*2 means neat stains visible on first day but extremely faint on second day

(c) Saliva

As discussed before, saliva stains are hard to be detected by naked eye due to its colourless [15]. Moreover, its fluorescence intensity is found to be very weak and very difficult to be detected by FLS compared to semen stains [15].

Besides, the absorbency of material became a factor that affects the detection for saliva stains, where saliva stains were very difficult to be detected when the stains were mostly absorbed into the fabric [4]. Vandenberg and Oorschot [4] reported that saliva stains on blue and white checked cotton weave were not visible when observed through 450nm and orange goggles. The maximum detectable dilution of saliva stains on white cotton using Polilight® reported was 1/16 [15].

Wawryk and Odell [13] reported that saliva stains on human skin was not fluorescence under high intensity LED, 370-480nm, and Poliray™, 450nm. However, it was reported that saliva stains on human skin was 100% sensitive to UV light, produced by Mineralight, 254nm, and Evident Products CE, 365nm, while 14% sensitivity when using Bluemaxx BM500 [21]. Spectra-Physics® Reveal™ laser has slightly better detection sensitivity compared to Lumatec Sperlite 400 for saliva stains [9].

The maximum dilutions of saliva stains on different materials detected by the stated FLS were summarized in **Table 8**.

(d) Urine

Literature on maximum detectable dilution of urine stains on different surfaces using FLS was limited. Vandenberg and Oorschot [4] reported that urine stains on white cotton was detectable with Polilight® but the serial dilution detection reported in the literature was only for blood, semen and saliva stains.

However, the maximum detectable dilution of urine stains on different surfaces, using Spectra-Physics Reveal laser and Lumatec Superlite 400, were reported by Seidl *et al*. [9], where their detection capabilities were comparable. Short UV light by Mineralight and 450nm light by Bluemaxx BM500 were able to detect urine stains on skin, with 71% and 14% sensitivity respectively [21].

There was also a test of the detection of urine stains on skin using different type of LED and Poliray® [13]. However, only higher intensity LED, LuxeonTM LED and Poliray® were reported to be successfully in detecting urine stains, with the same intensity with semen stains. These stains were undetectable by the mentioned FLS on the second day of experiment [13].

The maximum dilutions of urine stains on different materials detected by the stated FLS were summarized in **Table 9**.

Discussion

FLS was proven to be successful in detecting biological stains, such as blood, semen, saliva and urine. The best single wavelength and goggles combination for detecting most of the stains was 450nm wavelength with orange goggles [4]. Almost all types of biological stains on white cotton were visible with this combination of wavelength and goggles.

Most of the reported detection methods using FLS were observed through naked eyes. However, different examiners with the same combination of FLS and goggles could observe different colour for the same stains. For example, Carter-Snell *et al*. [21] reported that fluorescence of semen stains on skin were observed by different examiners with different colours when exposed to the same FLS and viewed with the same goggles. Moreover, some of the examiner failed to detect the stains [21]. Hence, there might be failure in detecting biological stains through human examiner with FLS during crime scene investigation. Besides, some fabrics would fluorescence when exposed to the FLS, such as white cotton would fluorescence at emission wavelength of 440-470nm with 340-410nm excitation wavelength and pink satin would fluorescence at emission wavelength of 570-620nm with 490-530nm excitation wavelength [22]. These fabrics' fluorescence would make the fluorescence of stains difficult to be detected. Hence, different fabric would need different wavelengths with different interference filters or goggles for the stain fluorescence to be successfully seen.

Table 8: Detectable saliva stains on different surfaces reported using FLS

FLS	Wavelength (nm) / Detection methods	Surfaces	Maximum visible dilution reported
Polilight®	450nm + Orange goggles [4,15]	2	1/16
		10,12,19	1
		3-9,11,13-18,20-27	–
Bluemaxx BM500	450nm + Orange goggles [14,21]	44	1
Mineralight	254nm [21]	44	–
Evident Products CE	365nm [21]	44	–
Lumatec Superlite 400	415nm + Orange goggles [9]	28	1/100
		29	**1/10**
		30	1
		31	1/100
		32	**1**
		25	1
Spectra-Physics Reveal laser	532nm + 532nm block goggles [9]	28	1/100
		29	**1/10**
		31	1/100
		32	**1**
		25	1/10

Code: Surfaces numbers referred to the surfaces numbered in Table 5
Bold dilutions mean strong or clear detection.
Non-bold dilutions mean weak detection.
"–" mean not stated in literature

Table 9: Detectable urine stains on different surfaces reported using FLS

FLS	Wavelength (nm) / Detection methods	Surfaces	Maximum visible dilution reported
Polilight®	450nm + Orange goggles [4]	2	–
	415nm + Yellow goggles [4]	2	–
	505nm + Red goggles [4]	2	–
Bluemaxx BM500	450nm + Orange goggles [21]	44	1
Mineralight	254nm [21]	44	–
Lumatec Superlite 400	415nm + Orange goggles [9]	1	1/100
		28	1/1000
		29	**1/10**
		30	1/100
		31	1/1000
		32	1/10
		25	1/100
		33	1/10
		34	1
Spectra-Physics Reveal laser	532nm + 532nm block goggles [9]	1	1/10
		28	**1/1000**
		29	**1/10**
		30	**1/100**
		31	1/1000
		32	**1**
		25	**1/100**
		33	1
		34	**1**
High Intensity LED (Luxeon^{TM} LED)	452.9nm/466.9nm + orange goggles [13]	44	*nv2*
Poliray®	450nm + orange goggles [13]	44	*nv2*

Code: Surfaces numbers referred to the surfaces numbered in Table 5
Bold dilutions mean strong or clear detection.
Non-bold dilutions mean weak detection.
"–" means not stated in literature
nv2 means neat stains visible on first day but not visible on second day

Future Developments

Background correction method [11,12], a multispectral imaging algorithm, showed improvement in detection of bloodstains. Hence, there might be improvement of detection of biological stains using multispectral imaging system compare to current methods. Multispectral imaging system is an imaging system that process images captured in different wavelength. Multispectral imaging system shows an important role for enhancing the images in many applications i.e. food industries [23], medical industries, printing [24] and forensic [11,12].

Besides, an automated imaging system would aid the crime scene investigator in detecting biological evidence. Since there would be different rate of detection of biological evidence by human observation, a computerized detection system with the aid of camera and FLS would give a more precise detection.

Further investigation for multispectral imaging system and automated detection system for detecting various types of biological stains can be done to improve the detection of biological stains using FLS.

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