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The adaptation of a 360° camera utilising an alternate light source (ALS) for the detection of biological fluids at crime scenes.

Article to be printed in colour.

Abstract

One of the most important and commonly encountered evidence types that can be recovered at crime scenes are biological fluids. Due to the ephemeral nature of biological fluids and the valuable DNA that they can contain, it is fundamental that these are documented extensively and recovered rapidly. Locating and identifying biological fluids can prove a challenging task but can aid in reconstructing a sequence of events. Alternate light sources (ALS) offer powerful non-invasive methods for locating and enhancing biological fluids utilising different wavelengths of light. Current methods for locating biological fluids using ALS's may be time consuming, as they often require close range searching of potentially large crime scenes. Subsequent documentation using digital cameras and alternative light sources can increase the investigation time and due to the cameras low dynamic range, photographs can appear under or over exposed. This study presents a technique, which allows the simultaneous detection and visualisation of semen and saliva utilising a SceneCam 360º camera (Spheron VR.), which was adapted to integrate a blue Crime Lite XL (Foster + Freeman). This technique was investigated using different volumes of semen and saliva, on porous and non-porous substrates, and the ability to detect these at incremental distances from the substrate. Substrate type and colour had a significant effect on the detection of the biological fluid, with limited fluid detection on darker substrates. The unique real-time High Dynamic range (HDR) ability of the SceneCam significantly enhanced the detection of biological fluids where background fluorescence masked target fluorescence. These preliminary results are presented as a proof of concept for combining 360º photography using High Dynamic Range (HDR) and an ALS for the detection of biological stains, within a scene, in real time, whilst conveying spatial relationships of staining to other evidence. This technique presents the opportunity to rapidly screen a crime scene for biological fluids and will facilitate simultaneous location and visualisation of biological evidence.

Keywords: Alternate light source; biological fluids; visualisation; 360-degree photography; high dynamic range; fluorescence

Highlights

- 360º camera adapted using Alternate Light Source to visualise trace evidence
- This method successfully visualised and documented semen and saliva
- Semen and saliva harder to visualise on dark cotton surfaces using Blue Light
- Preliminary results demonstrate successful proof of concept for combined method
- This technique could provide a more efficient method than current approaches

1. Introduction

Biological fluids, such as blood, semen, saliva, vaginal secretions and urine, are a commonly encountered evidence type that can be recovered at crime scenes. They serve as an invaluable evidence type given that they contain valuable DNA evidence that may be used to identify individuals present at the scene, including both suspect and victim. Identifying the location and distribution of biological staining within a crime scene is crucial to the investigation as the location and identity of the fluid can aid Forensic Investigators (FI) in reconstructing a sequence of events and determining what may have occurred at the scene [1]. Due to the ephemeral nature of this type of evidence, it is fundamental that the evidence is documented extensively and recovered quickly and efficiently. Locating biological fluids can prove a challenging task for FI's as many stains are invisible to the naked eye or are similar in appearance to other extant substances.

An Alternate Light Source (ALS) typically allows the selection of different wavelengths of light to help visualise evidence, otherwise invisible to the naked eye, based on the response received from the object of interest. ALS's offer powerful methods that can allow the enhancement and presumptive detection of trace evidence likely to be present at crime scenes [2] and are one of the simplest methods available for the detection of biological fluids [3]. Owing to both their simplicity and non-destructive or non-invasive nature they have been extensively utilised in criminal investigations to aid FI's in determining the location of trace evidence at crime scenes, particularly where limited sample quantities are exhibited [1, 4].

Once visualised, it is integral that the evidence is thoroughly documented in a manner that captures its distribution and location as it was at the time of the investigation. Digital photography allows the FI to document both the scene and the evidence and present it to a judge and jury in a courtroom in a simple and detailed manner [5]. Standard digital cameras have a lower dynamic range than the human eye and as a result photographs can appear under or overexposed in comparison. Evidence that has been enhanced using ALS's needs to be efficiently recorded as seen by the FI. Where ALS photography is utilised, fluorescence filters can be fitted over the existing camera lens to block the excitation wavelength of light and allow the camera to capture a response from the target substrate [6]. The use of an ALS can increase the time taken to process the crime scene. 360^{o} photography can capture a full panorama of a scene in one scan, conveying spatial relationships of evidence within the scene, ensuring the entire scene is captured rather than only those items deemed relevant at the time by the FI.

Current methods for utilising ALS techniques require the FI to search the crime scene at close range, which can be a time consuming task depending on the complexity of the environment. Issues may arise during close range searching, particularly where large crime scene environments are concerned, whereby the FI could be searching for long periods of time without any indication as to where biological fluids could be present. Once the stained location is determined, the evidence will be documented and photographed accordingly, further extending the investigation time prior to the evidence having been collected and analysed. Current methods for photographing a response from biological fluids when using an ALS require the FI to select the correct exposure in order to successfully capture a (fluorescent) response. This process will have to be repeated for multiple biological stains, adding further time onto the investigation process. 360^{o} photography with HDR can capture a complete 360^{o} view of an environment in addition to accounting for multiple exposures. Utilising a system which integrates an ALS within 360^{o} HDR photography could not only allow the detection of biological fluids at larger crime scenes, but could dramatically reduce the time it takes to identify, document, collect and analyse the evidence.

2. Method

In line with ethical requirements of the host institution and in accordance with health and safety procedures, human semen was obtained from one male donor, aged 26. Human saliva was obtained from a female donor aged 24. Biological fluid samples were collected into separate 100 ml Thermo Scientific™ Sterilin™ Polystyrene Containers and labelled accordingly. All biological fluid samples were collected on the morning of the study and were immediately stored in a fridge at 3^{o}C until required. White cotton, dark blue cotton, HP premium matte polypropylene white plotter paper (140 g/m^2), and coloured cardboard (160g/m^2; red, orange, yellow, green, blue and violet in colour) were utilised as the substrates for fluid deposition. The white cotton, dark blue cotton and white plotter paper substrates were cut into approximate 10 cm x 10 cm square swatches and the coloured cardboard substrate was cut into approximate 5 cm x 5 cm square swatches.

Using Biohit Proline® automated pipettes, 5, 50, 100, 150, 200 and 250 µL of the biological fluid were deposited onto each substrate type. The pipette was held directly above the substrate and the biological fluid deposited at a 90^{o} angle to the substrate. A series of between 1 and 4 drops of biological fluid were deposited onto multiple swatches as shown in Figure 1. For the coloured cardboard swatches, one single drop of each biological fluid was deposited. Samples were left to dry under ambient conditions (approximately 18^{o}C) for 24 hours.

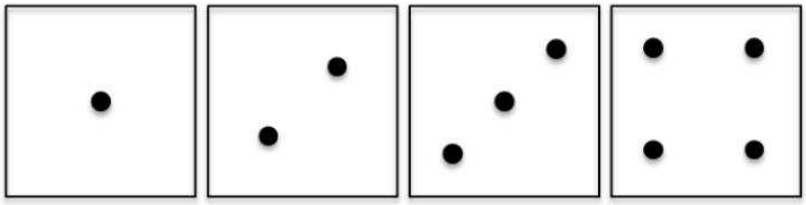


Figure 1: Drops of biological fluid deposited onto swatches

A specialist 'trauma room' at the host institution was utilised for this investigation as it provided an environment, which limited contamination from other biological fluids, and allowed for complete darkness. Walls in this room were covered with lining paper to remove the reflectivity and to ensure that the walls were more representative of common household environments. All swatches were adhered to the wall lining paper using double-sided sticky tape, in the approximate centre of one wall. The order with which each swatch was adhered to the wall was determined using a random number generator in Microsoft Excel.

The environment was illuminated using a Crime Lite XL (420-470 nm) (Foster + Freeman Ltd.) and photographed using a SceneCam 360^{o} camera (Spheron VR). A Crime Lite XL was held above and behind the camera lens as shown in Figure 2. The camera was initially positioned 30 cm away from the swatches. The camera was calibrated according to the manufacturers instructions (Spheron SceneCam User Manual, 2007).

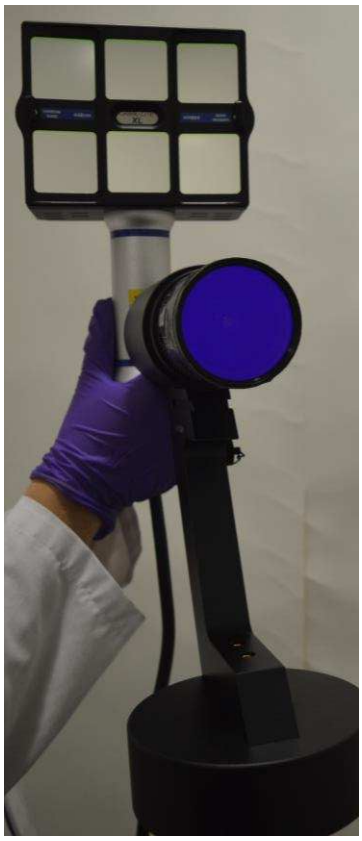


Figure 2: The Crime Lite XLs position in relation to the SceneCam.

A 495 nm (GG495) longpass camera filter (62 mm) was adhered, using Duct Tape™, over the existing fisheye lens on the 360º camera, to allow induced fluorescence to be observed (Figure 3).

Figure 3: GG495 camera filter attached to the already existing fisheye lens of the SceneCam

This process was repeated for 60, 90, 150 and 300 cm working distances, for each substrate and biological fluid type. The resulting panoramas were uploaded into the complimentary SceneCenter software. No photographs were enhanced or treated with Photoshop or any other digital image manipulation software.

2.1 Detection of Biological Fluids

The panoramas were initially monitored to determine whether the ALS and 360º camera combination could detect any biological staining on the four substrate types. Once it had been established that each of the biological fluids could be successfully located using the ALS and

camera combination, the accuracy of the technique was investigated using the following approach.

Ten participants; 4 male and 6 female, aged between 26 and 44 years of age, were recruited from the host institution. Participants were briefed on the aims of the investigation and were asked to sign a consent form in line with the ethical requirements of the institution. Participants were provided with an answer booklet, which had each numbered panorama and the distribution of the substrate swatches (Figure 4). Participants were required to replicate a pattern of biological fluid drops corresponding to the swatches in the 360º panoramas. Participants were told not to draw anything that was not circular in shape and were informed that they could use the High Dynamic Range (HDR) in the software to increase or decrease the light intensity to aid the visualisation of the biological fluids. The panorama order was randomised and the default titles removed and replaced with numbers.

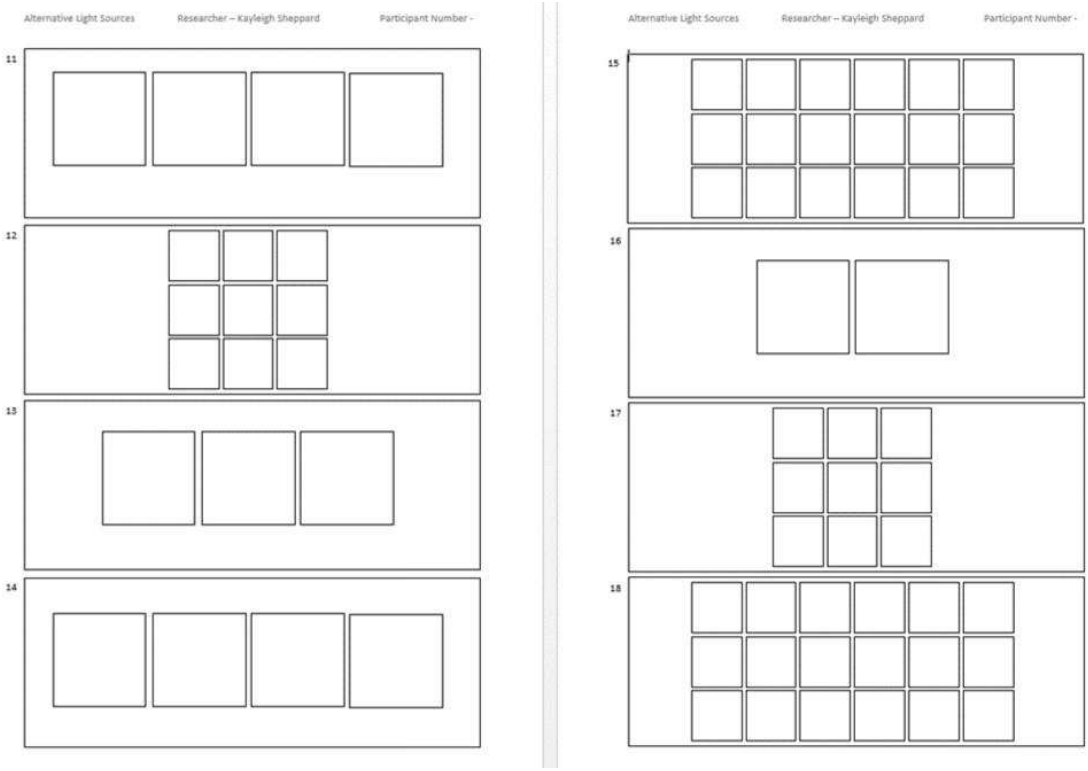


Figure 4: Answer booklet for participants to complete

The total number of drops identified by each participant was calculated by counting the number of drops they had drawn.

3. Results and Discussion

This is the first report demonstrating the successful location and visualisation of biological fluids at small volumes using a 360º camera system adapted using an alternate light source.

The location and documentation of semen and saliva using the blue Crime Lite XL and 360º camera technique on each substrate type are discussed in turn. Where contrast of biological stains were observed this was achieved using the 455 nm excitation wavelength and a 495 nm (GG495) longpass camera filter (62 mm).

3.1 White Cotton

The semen stains deposited onto the white cotton substrate appeared barely visible when examined under natural light. Using the Blue Crime Lite XL at 455 nm excitation wavelength the seminal fluid demonstrated fluorescence, which is consistent with recommended best practice [7, 8, 9]. The fluorescence was successfully documented by the 360º camera as shown in Figure 5.

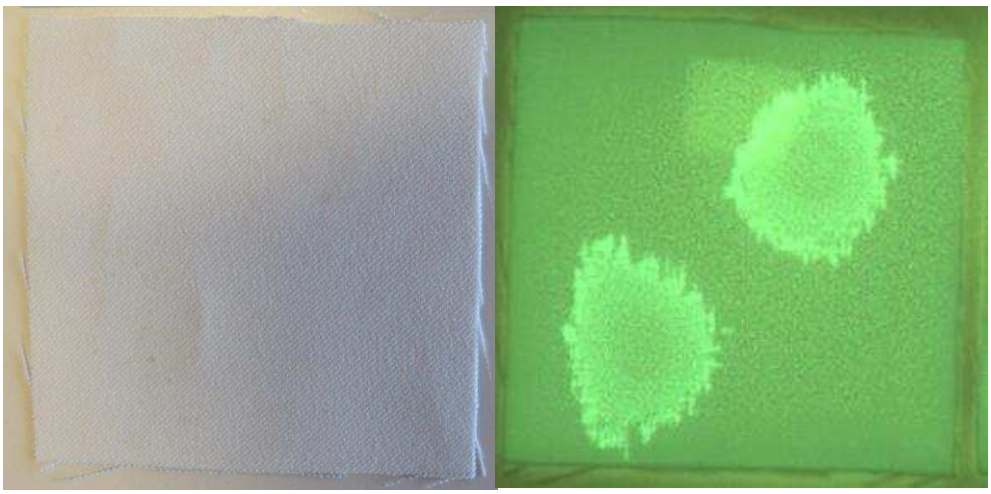


Figure 5: 200 µL Semen staining on white cotton swatch 10 cm x 10 cm
Left: Semen exposed to natural light. Right: Semen exposed to a blue Crime Lite XL

The camera system and ALS technique was able to successfully detect semen stains on the white cotton substrate to volumes as small as 5 µL. This was possible for all of the distances studied. Figure 6 demonstrates the seminal fluid fluorescence detected by the 360º camera and Blue Crime Lite XL for all volumes at 30 cm and 90 cm distances.

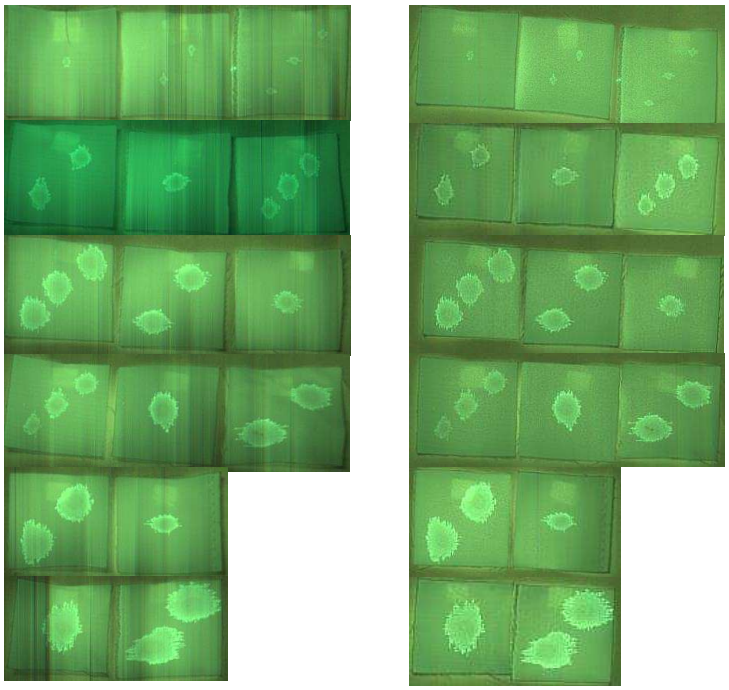


Figure 6: All volumes (from top to bottom), 5, 50, 100, 150, 200 and 250 µL – seminal fluid successfully detected on white cotton using a blue Crime Lite XL at 30 cm (left), 90 cm (right)

Similarly to semen, saliva appeared barely visible to the naked eye under natural lighting, but was successfully visualised and documented for some of the samples of saliva using a Blue

Crime Lite XL and 360º camera. Recommended best practice utilised 455 nm such as that which the blue Crime Lite XL provides [10]. The fluorescence demonstrated by a saliva stain is demonstrated in Figure 7.

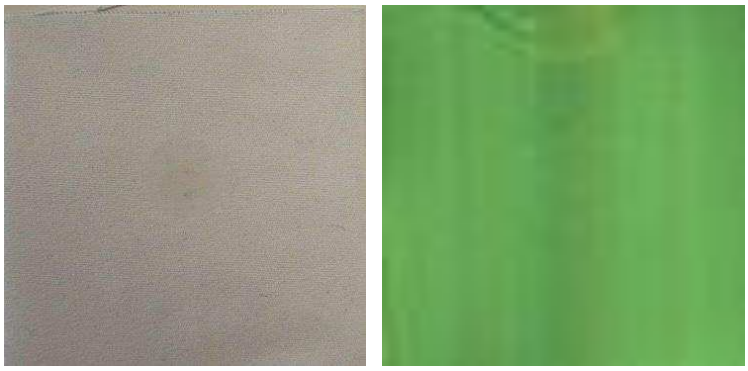


Figure 7: 200 µL Saliva staining on a white cotton swatch 10 cm x 10 cm. Left: Saliva exposed to natural light. Right: Saliva exposed to a blue Crime Lite XL

Saliva staining was successfully located in the majority of cases using a blue Crime Lite XL, but visualisation was only possible with larger volume stains as shown in Figure 8. This was consistent with results observed by Camilleri et al [11]. Smaller volume stains were more difficult or impossible to detect, which could be attributed to the lack of solid particles within the saliva sample [1,3]. In addition, detection of saliva on the white cotton substrate was difficult due to the porous nature of the surface type. As a result, the saliva was absorbed into the material rather than drying on the surface, leaving little surface fluorescence. The fluorescence from the biological fluid could also have been masked by background fluorescence from the white cotton material. When subjected to blue or ultra-violet light (UV), white materials can exhibit fluorescence due to the presence of naturally occurring organic compounds within the material, or optical brighteners present in detergents [7]. Background fluorescence from the substrate can mask the target fluorescence, increasing the difficulty in detecting the biological fluid [12].

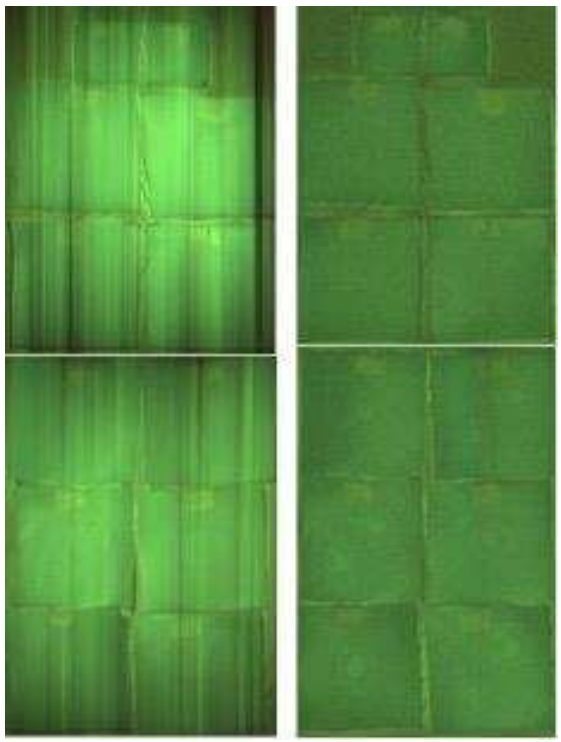


Figure 8: All volumes (from top to bottom), 5, 50, 100, 150, 200 and 250 µL – larger saliva stains successfully detected on white cotton using a blue Crime Lite XL at 30 cm (left), 300 cm (right)

3.2 Dark Blue Cotton

Semen was detected under natural light immediately after deposition on the dark blue cotton. Following a 24-hour drying period, the biological staining had dried, and only some staining was still visible under natural light. These stains could be successfully located and documented using a blue Crime Lite XL and 360° camera, as shown in Figure 9.

Figure 9: 200 µL semen staining on a dark blue cotton swatch 10 cm x 10 cm
Left: Semen exposed to natural light. Right: semen exposed to a blue Crime Lite XL

Unlike the white cotton, which can contain naturally fluorescent organic compounds, the dark cotton was less likely to contain these substances and mask fluorescence from the semen stains. In this study, the dark cotton was not found to fluoresce itself, but this material presented other problems in the location and detection of the semen stains. The dark material could absorb excited and emission fluorescence from some of the biological stains, making them less visible. These results were consistent with research conducted by Kobus et al [7] and Fiedler et al [13] which reported a high degree of difficulty in detecting seminal fluid on materials, which were dark in colour, highly absorbent, or made of material which itself is naturally fluorescent, such as white cotton.

As shown in figure 9 (right), not all of the biological fluid droplets were consistent in terms of their visibility using the blue Crime Lite XL. This was likely to have been due to incomplete deposition, perhaps due to air bubbles produced during deposition. However, those stains that could be detected by the camera were detectable up to a maximum distance of 300 cm away from the staining, as shown in figure 10. As the camera and ALS moved further away from the staining, the semen stains became harder to detect and proved more challenging to document.

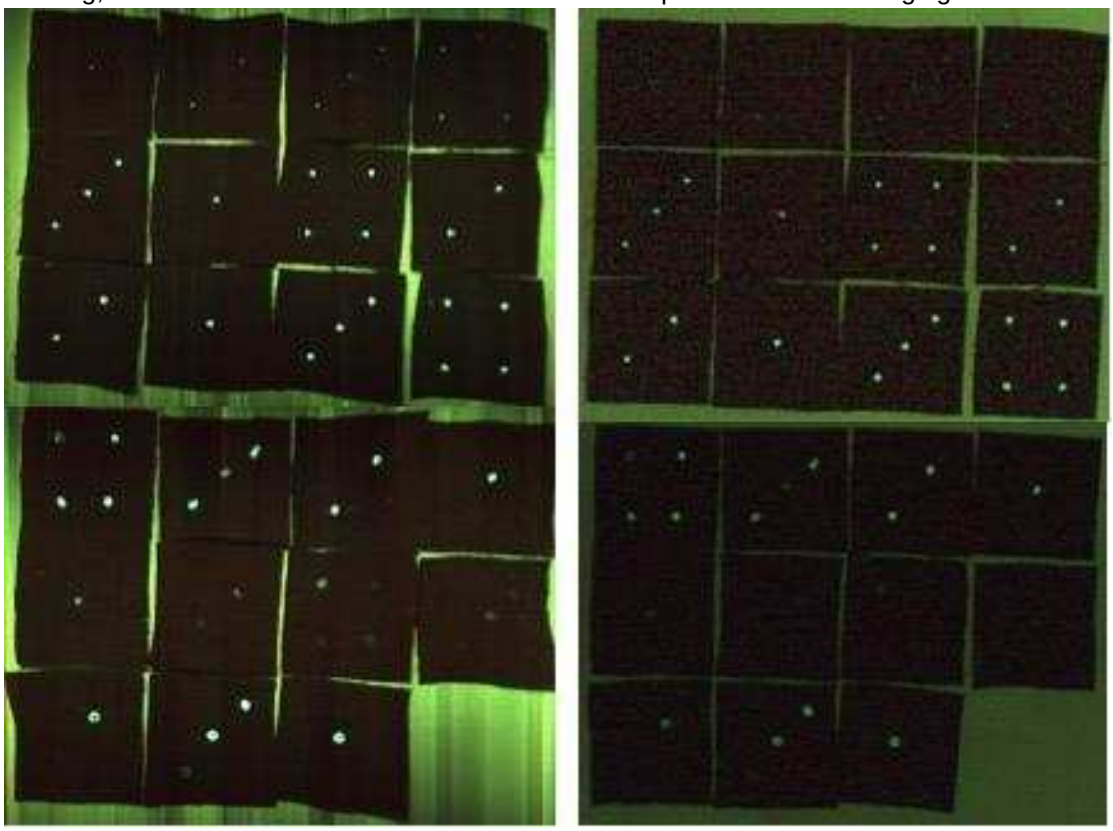


Figure 10: All volumes (from top to bottom), 5, 50, 100, 150, 200 and 250 µL – seminal fluid successfully detected on dark blue cotton using a blue Crime Lite XL at 30 cm (left), 300 cm (right)

Saliva, which is virtually colourless in composition, proved more difficult to detect on the dark blue cotton substrate than semen. In many cases, the saliva stains were not enhanced using the ALS, and remained invisible to the naked eye, as shown in Figure 11. The saliva stains exhibited little response or fluorescence. This could be attributed to the absorbent nature of the substrate whereby saliva was absorbed further into the material whilst drying, as opposed to drying on the surface of the substrate [3].

Figure 11: 200 µL Saliva staining on dark blue cotton swatch 10 cm x 10 cm
Left: Saliva exposed to natural light. Right: Saliva exposed to a blue Crime Lite XL

The majority of the saliva stains were impossible to detect on the dark blue cotton fabric using a blue Crime Lite XL for all volumes and distances examined, as shown in Figure 12, with only one or two drops actually being detected. In these few cases, the fluorescence demonstrated by the stains was very low intensity, which made the stains more difficult to detect. The limited detection of saliva on this substrate could be attributed to the porous nature of the material, whereby the saliva absorbed into the fabric, and due to the lack of solid particles within the saliva, as previously described in 3.1 [3, 11].

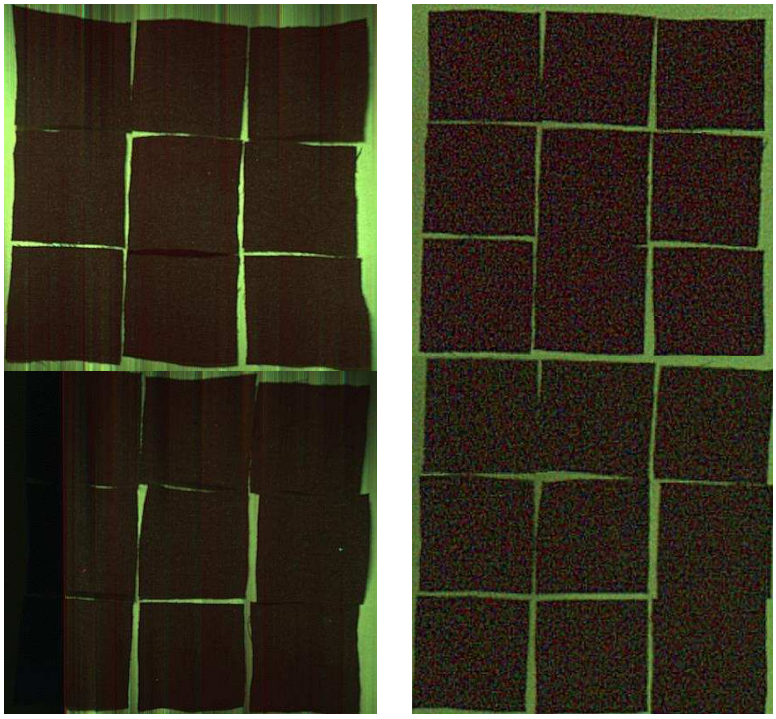


Figure 12: All volumes (from top to bottom), 5, 50, 100, 150, 200 and 250 µL unsuccessfully detected for saliva on dark blue cotton using a blue Crime Lite XL at 30 cm (left), 300 cm (right)

The samples of saliva were rapidly absorbed into the white and dark blue cotton substrates once deposited. In some of the tests conducted on these materials the biological fluid was undetectable, or the fluorescence observed was weak in intensity. The absorption of the biological fluid into the substrate inhibited the ability to detect the fluorescence of the fluid in

some cases. The smaller volumes of biological fluid deposited had a tendency to sit on the surface of the substrate without being absorbed, making the stains easier to detect. In contrast, the semen samples were easier to detect on the same substrates, and this could have been attributed to the higher viscosity of the seminal fluid, which allowed the fluid to sit on the surface of the substrate once deposited, as shown in Figure 9. This is consistent with results demonstrated by Vanderberg and Oorschot [9]. Where a fluorescent response was not observed the presence of a biological fluid cannot be excluded and further testing would be required [7].

3.3 White Plotter Paper

The semen stains deposited onto the white plotter paper substrate were visible when examined under natural light. When subjected to a blue Crime Lite XL, the seminal fluid demonstrated high intensity fluorescence, which was successfully documented using the 360º camera system, as shown in Figure 13.

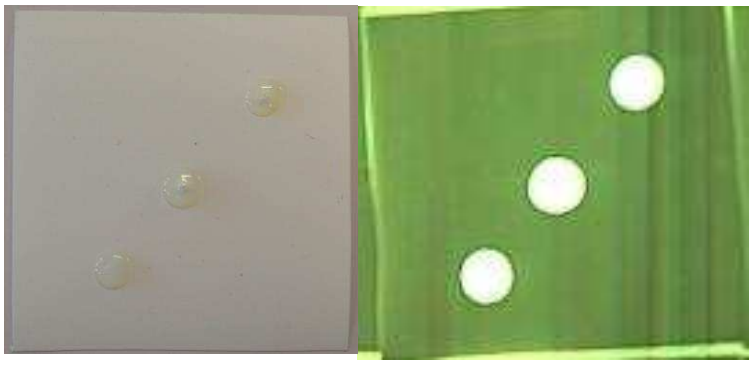


Figure 13: 200 µL Semen staining on white plotter paper 10 cm x 10 cm
Left: Semen exposed to natural light. Right: Semen exposed to a blue Crime Lite XL

The camera system and ALS technique was able to successfully detect semen stains on the white plotter paper to volumes as small as 5 µL. This was possible for all of the distances studied. The fluorescence observed by the semen on the white plotter paper substrate appeared to exhibit high intensity fluorescence. Figure 14 demonstrates the seminal fluid fluorescence detected by the 360º camera and blue Crime Lite XL for all volumes at 30 cm and 300 cm distances.

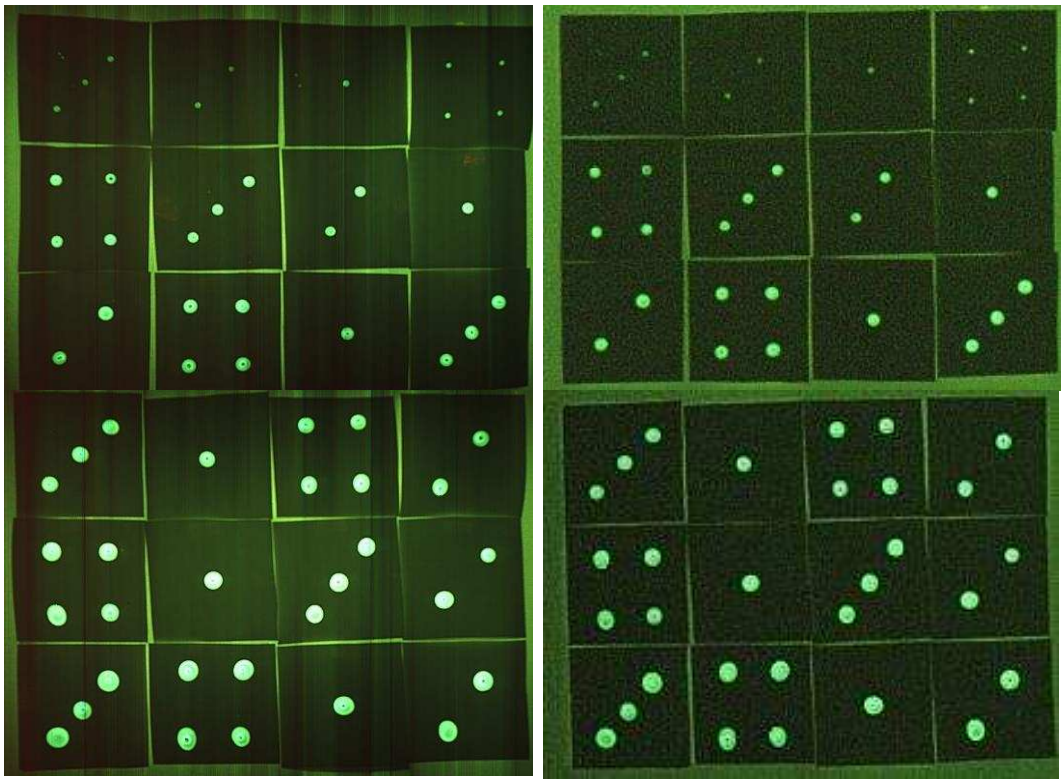


Figure 14: All volumes (from top to bottom), 5, 50, 100, 150, 200 and 250 µL – seminal fluid successfully detected on white plotter paper using a blue Crime Lite XL at 30 cm (left), 300 cm (right)

Saliva deposited onto the white plotter paper substrate was visible under natural light, but was visualised more easily using a blue Crime Lite XL. The saliva stains were successfully located and documented using the 360^{o} camera, as shown in Figure 15.

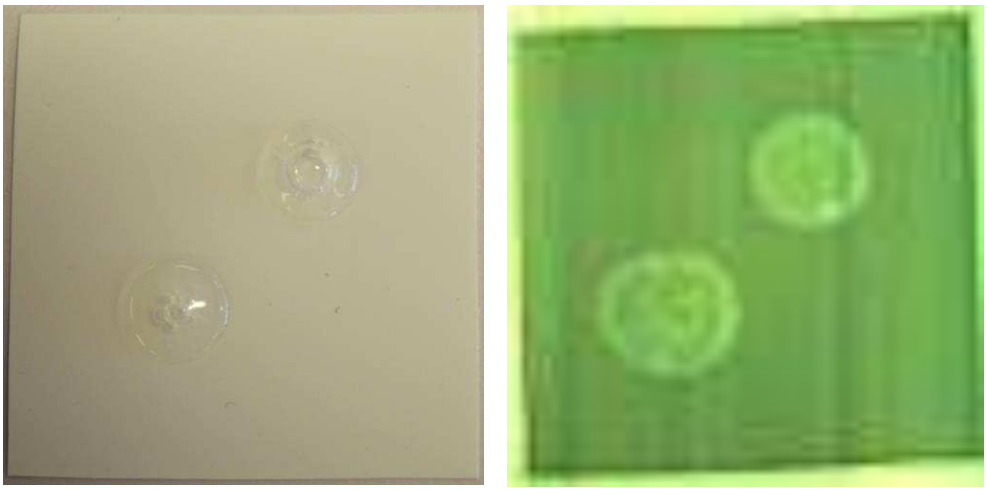


Figure 15: 200 µL Saliva staining on White Plotter paper 10 cm x 10 cm.
Left: Saliva exposed to natural light. Right: Saliva exposed to a blue Crime Lite XL

The camera system and ALS technique was able to successfully detect saliva stains on the white plotter paper to volumes as small as 5 µL, although the smaller volumes were more difficult to visualise and document with the 360^{o} camera system. Documentation of the smaller

volume stains became more difficult as the working distance increased. Figure 16 demonstrates the saliva fluorescence detected by the 360º camera and blue Crime Lite XL for all volumes at 30 cm and 90 cm distances.

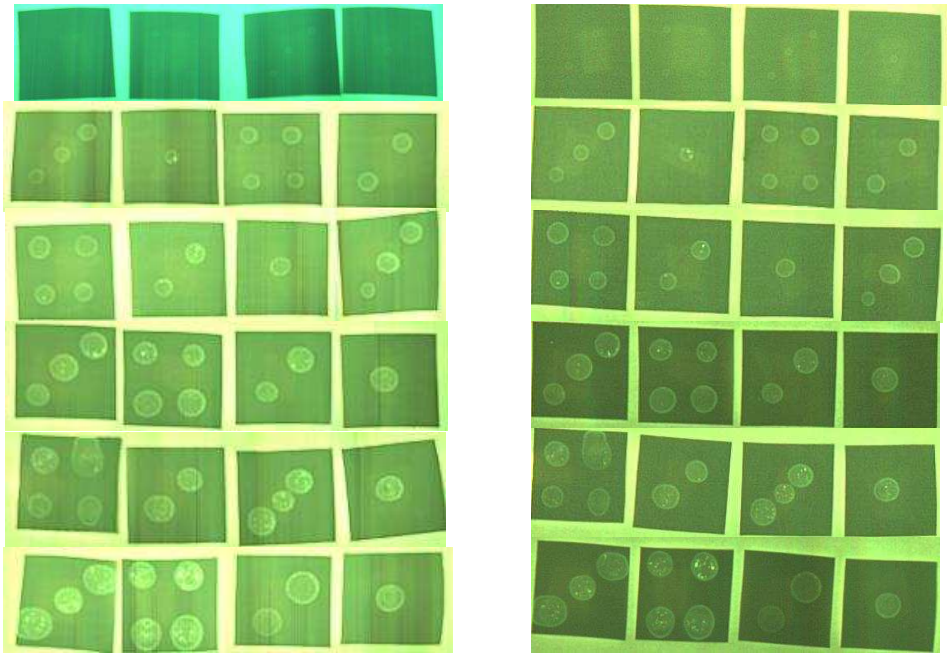


Figure 16: All volumes (from top to bottom), 5, 50, 100, 150, 200 and 250 µL – saliva successfully detected on white plotter paper using a Blue Crime Lite XL at 30 cm (left), 90 cm (right)

For the saliva stains, the identified fluorescence was concentrated around the outer edges of the saliva stain with very little fluorescence in the centre of the stain. Saliva exhibited low intensity fluorescence when compared to the fluorescence exhibited by the semen on the white plotter paper substrate, as shown in Figure 17. The fluorescence observed by the semen stains occurred across the entirety of the stain, which was likely to be attributed to the presence of conjugated choline and flavin proteins within the seminal fluid [7]. Knowledge about the different responses biological fluids have to certain wavelengths of excitation light can aid in estimating but not determining between semen and saliva fluids [14]. However, the definitive nature of a fluorescent area cannot be determined solely through visual inspection and any fluorescent areas will require further confirmatory testing to ascertain the identity of the fluid [4, 15].

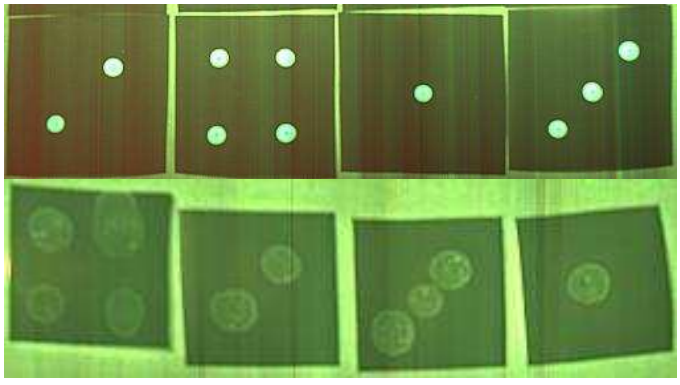
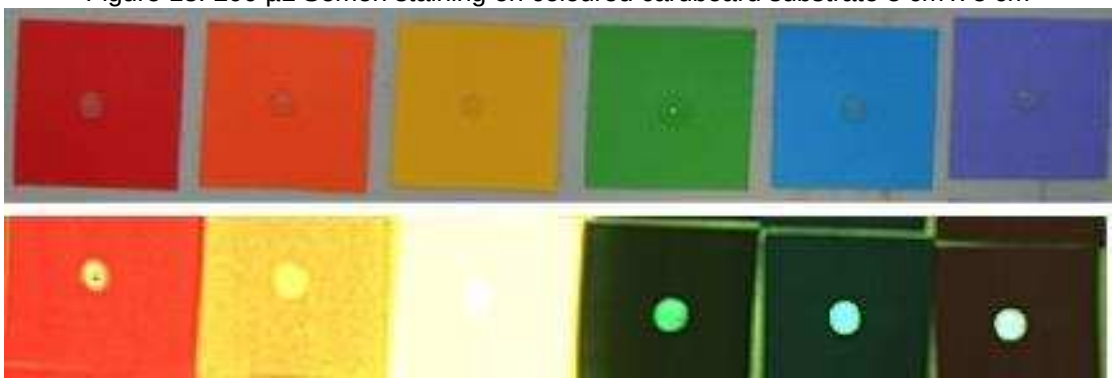


Figure 17: 200 µL stains on white plotter paper substrate exposed to a blue Crime Lite Top: Semen. Bottom: Saliva

3.4 Coloured Cardboard

The semen stains deposited onto the coloured cardboard substrate were visible when examined under natural light. When subjected to a blue Crime Lite XL, the seminal fluid demonstrated high intensity fluorescence, which was successfully documented using the 360º camera system, as shown in Figure 18.

Figure 18: 200 µL Semen staining on coloured cardboard substrate 5 cm x 5 cm



Top: Semen exposed to natural light. Bottom: Semen exposed to a blue Crime Lite XL

In some cases the yellow cardboard produced limited results, particularly for the smaller volumes, where the background fluorescence from the yellow cardboard masked the fluorescence from the semen stains. In these cases the High Dynamic Range (HDR) of the SceneCam enabled fluorescence previously masked by the background to be visualised successfully. The intensity of the light source on the stains did have an effect on the fluorescence detected by the 360º camera system. However, the unique HDR capabilities of the optical system allowed visualisation of the biological fluids even when this appeared to be masked by background fluorescence from the substrate, as shown in Figure 19 (Top). Photographing fluorescence from biological fluids using a digital camera can prove difficult when background fluorescence is present due to the masking, and may require a series of different photographs to be taken at multiple exposures to try and reduce the fluorescent response from the background and enhance the target fluorescence. In this study, the unique addition of the HDR resulted in noticeably greater contrast between the staining and the background, allowing greater visibility of the stains, as shown in Figure 19. The HDR controls within the complementary software allows the luminance levels to be increased or decreased without digitally altering or manipulating the image, as the camera accounted for all the different light levels and exposures as it scanned at the time of image acquisition.

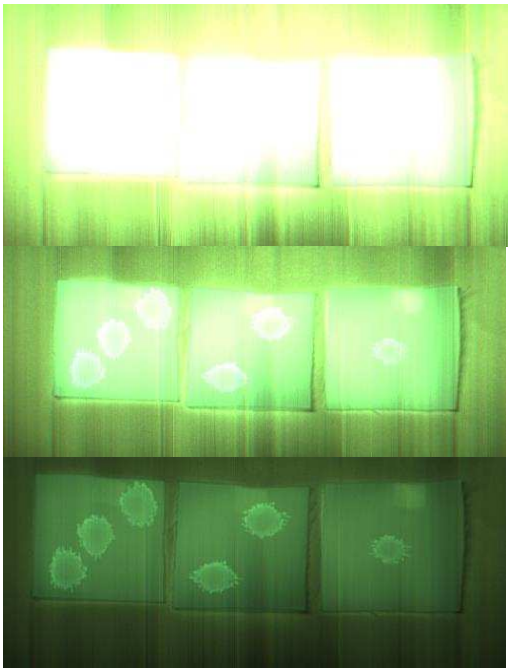


Figure 19: Real time HDR applied to the detection of semen stains on white cotton. Top: Default exposure with masked fluorescence. Middle: Lowered exposure showing semen fluorescence. Bottom: Lowered exposure further to fully observe the shape and contrast of the semen stains.

The majority of the semen stains deposited onto the coloured cardboard substrate were successfully visualised and documented by the Crime Light XL and 360º camera system. This was successful for most volumes at all distances examined, as shown in Figure 20. At greater distances the smaller volumes, such as 5 µL, became more difficult or impossible to detect.

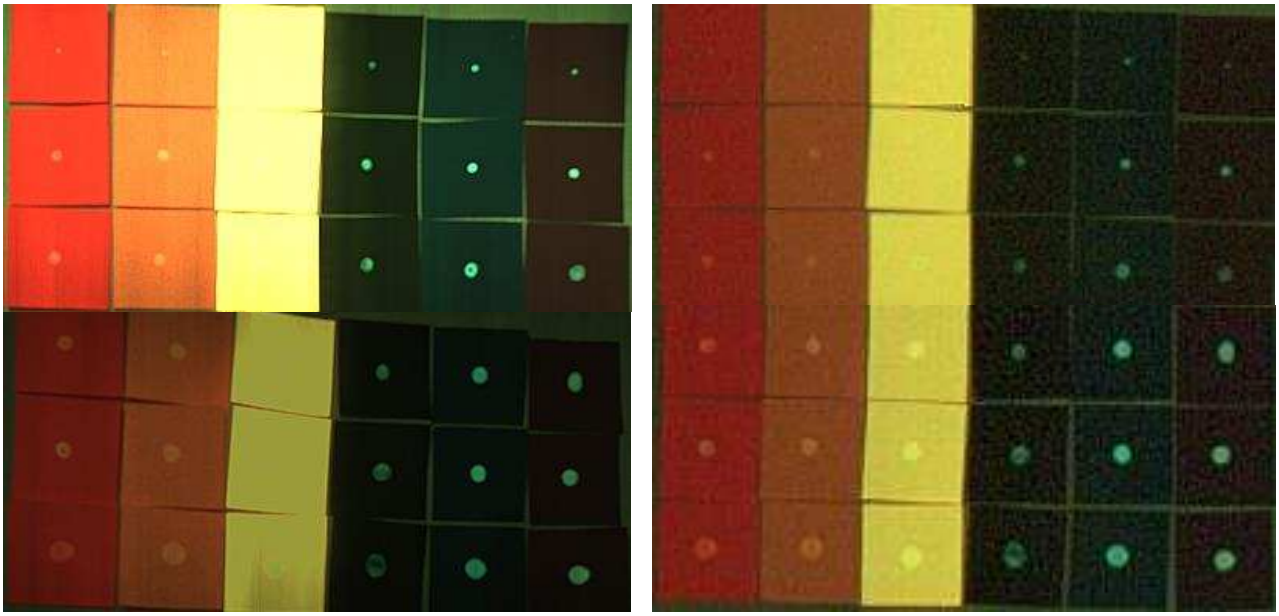


Figure 20: All volumes (from top to bottom), 5, 50, 100, 150, 200 and 250 µL – seminal fluid successfully detected on coloured cardboard using a blue Crime Lite XL at 30 cm (left), 300 cm (right)

The 360º camera and light source were moved further away from the stained swatches to determine whether the distance had any effect on the ability of the camera to document the staining. The distance of the camera and light source technique had no effect on the resultant fluorescence of the biological staining, but the larger distances meant the 360° camera could not document some of the smaller volumes (5 µL and 50 µL) of biological fluids successfully. The resolution of the camera will become a limiting factor for the detection of the biological staining as the camera and light source distance increases. Further investigation can be conducted to determine the effects that the resolution will have on the documentation of the biological fluids. As the camera moves further away from the target staining the area covered by a single pixel becomes larger, limiting the detail that can be captured (Figure 21). De Forest et al [16] identified that the result of zooming in on an image compromised the ability to resolve smaller volume stains. In this study the camera resolution did not compromise the ability to locate the staining due to the limit of the room size of 300 cm. At significantly greater distances however, it is expected that the resolution will become a limiting factor for the successful documentation of biological staining. This study has demonstrated that the 360º camera and alternative light source combination could successfully detect and document biological staining on different substrates at different distances from the substrate. As a result, this technique could provide a more effective method for locating biological fluids than current methods, whereby close range searching is conducted. This technique could eliminate the need for close range blind searching of a crime scene and direct an investigators attention to target staining more quickly. The opportunity to rapidly screen a crime scene for biological fluids will facilitate simultaneous location and visualisation of evidence.

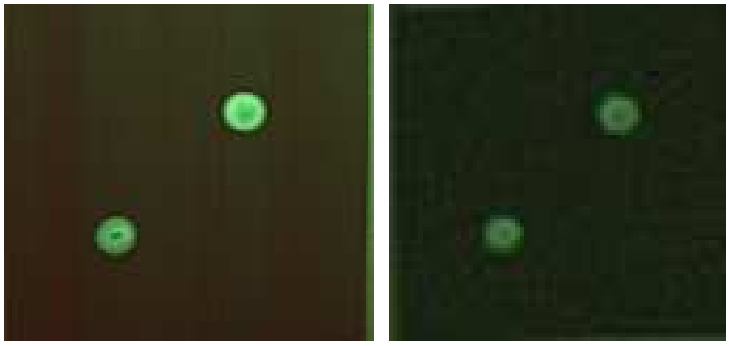


Figure 21: 100 µL Semen stains on white plotter paper substrate exposed to a blue Crime Lite XL. Resolution difference: (Left) 30 cm. (Right) 300 cm camera distance from seminal fluid stain

At a greater working distance, the intensity of the light source may become a limiting factor. A high powered light source will be more likely to induce a fluorescent response from staining at greater distances than a low powered light source. As a result the intensity and power from the light source must be considered before embarking on this work. In this study, the intensity of the light source was inconsequential and did not affect the ability to induce a fluorescent response from the biological staining. The Crime Lite XL provides 96 high intensity LEDs that, in this case, was sufficient for illuminating an entire internal wall at a distance of up to 3 metres. Due to the high intensity illumination provided by the Crime Lite XL, some ambient lighting within the environment did not prove problematic. Some ambient lighting was present during this investigation, whereby lighting from a laptop connected to the camera was present and lighting from the adjacent room. These other light sources did not seem to affect the enhancement of the biological staining, and as a result we can conclude that it is not essential

to block out all light within the scene. This provides significant benefits over methods that require complete darkness in order to successfully detect biological staining. De Forest et al, [16] came to the same conclusion where it was not necessary to block out all ambient light from a scene.

3.5 Other artefacts

The camera system adapted with the ALS was capable of detecting other artefacts in addition to the biological fluids on the materials, as shown in Figure 22. Fibres and other small particles were enhanced by the light source and produced a fluorescent response. As a result this technique, with appropriate lighting and filters, could also be used as a screening method for other types of evidence, including hairs and fibres, in addition to biological fluids. De Forest et al, [16] found that the light sources used in their study also detected other artefacts such as fibres on the material.

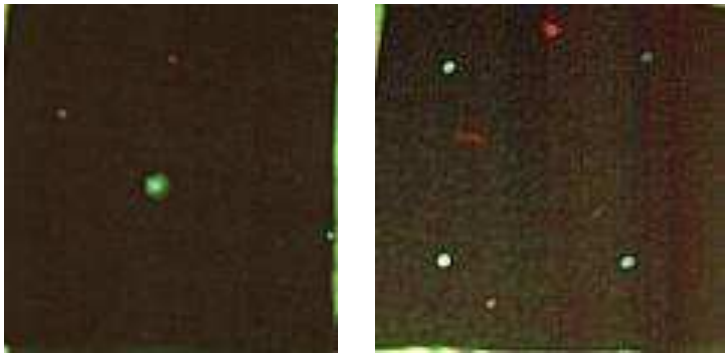


Figure 22: Artefacts such as fibres identified on the substrate.

3.6 Participant Detection of Biological Fluids

The number of drops of seminal fluid drawn by the participants can be found in Table 1.

Table 1: The number of seminal fluid drops identified on each of the substrates by each participant

Substrate Type	**Participant Number**										**Total Number of Drops Deposited**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
	Number of seminal fluid drops identified by participants										
White Cotton	99	99	99	99	99	99	99	99	99	99	99
Dark Blue Cotton	270	264	263	238	271	305	249	252	258	259	280
White Plotter Paper	300	300	300	300	300	300	304	300	300	300	300
Coloured Cardboard	176	174	173	178	175	180	173	160	172	162	180

The results in table 1 suggest that semen can be located and visualised on white cotton with a high degree of accuracy, given that all 10 participants identified 99 seminal fluid drops on the white cotton substrate (100% of seminal fluid drops identified given that 99 drops were deposited in total). For the white plotter paper substrate, 9 participants identified 300 drops of seminal fluid and 1 participant identified 304 drops. A total of 300 drops of seminal fluid were deposited onto the plotter paper and so participant 7 identified 4 more drops than were originally deposited. Despite the apparent high level of accuracy evidenced by the remaining participants, participant 7 could have identified artefacts on the substrate which were not the target biological fluid. The authors were not concerned by this result, given that the technique had been able to locate and visualise the known semen samples, and accept that during casework, further analysis of any located sample would have to commence in order to identify the source of the biological fluid.

A reduced level of accuracy was exhibited on the dark blue cotton and cardboard substrates compared to the white cotton and white paper substrates. Participants detected between 238 and 305 drops of the 280 drops of seminal fluid that were initially deposited onto the dark blue cotton substrate. Participant 6 identified 25 more drops than were initially deposited and this could be attributed to artefacts present on the substrate, such as fibres or other particles, which fluoresced.

Participants' detection of seminal fluid drops on the coloured cardboard ranged from 160 drops to 180 drops out of the 180 seminal fluid drops initially deposited. Just one participant identified all 180 drops of seminal fluid. The reduction in the level of accuracy was attributed to the substrate type. The yellow cardboard in particular demonstrated background fluorescence which masked the fluorescence from the semen stains, making them harder or impossible to detect. In addition, the increased working distances made the smaller volumes harder to detect and thus some participants were not able to detect the seminal fluid in these cases.

The number of drops of saliva drawn by the participants can be found in Table 2.

Table 2: The number of saliva drops identified on each of the substrates by each participant

Substrate Type	**Participant Number**										**Total Number of Drops Deposited**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
	Number of saliva drops identified by Participants										
White Cotton	32	31	20	35	37	26	31	15	30	24	90
Dark Blue Cotton	3	5	21	6	4	100	7	0	1	3	180
White Plotter Paper	180	178	178	178	180	180	178	176	180	178	180
Coloured Cardboard	166	150	166	151	166	178	165	158	163	119	180

In comparison to semen, considerably less accuracy was demonstrated by participants during the location and visualisation of saliva. Four participants were able to identify all 180 saliva drops on the white plotter paper substrate, with 5 participants missing 2 drops initially deposited, and one participant failing to detect 4 drops (1.11% missed), as shown in table 2. The majority of participants identified >87% of the total number of drops initially deposited on coloured cardboard. Participant 10 only managed to identify 66 % of saliva drops on the coloured cardboard, which could be attributed to its colour, and the yellow substrate demonstrating background fluorescence, masking the fluorescence of the saliva. In addition, saliva can be more difficult to detect due to a less intense fluorescent response caused by a lack of solid particles within the biological fluid [1,3].

The level of accuracy associated with locating and visualising saliva stains on white cotton was significantly reduced, with only 33 % of the total drops deposited being successfully identified. The reduced level of accuracy associated with the detection of saliva on white cottons is likely to have been due to the inherent fluorescence observed by the substrate, thus masking the fluorescence from the saliva [7, 11].

Very few participants were able to detect saliva stains on dark blue cotton. This could be attributed to the porous nature of the substrate whereby the saliva was absorbed into the substrate rather than drying on the surface, leaving little surface reflectance [3, 7, 12]. The difficulty in detection of saliva could also be due to the very weak nature of saliva fluorescence [11].

The results of this research have demonstrated a variation in the ability to locate and visualise semen and saliva on a variety of substrates using a non-destructive technique; 360° photography combined with an alternate light source. Further investigation observing a broader range of substrates is planned to determine the optimum conditions and limitations of this combined technique and its applications for casework, particularly in the presence of alternative agents, which may also fluoresce, and therefore introduce false positive results. In addition, the authors recommend the investigation of other biological fluids, such as vaginal secretions and urine, to determine the optimum conditions for their successful location and visualisation.

4. Conclusion

The results of this research have demonstrated a variation in the ability to locate and visualise semen and saliva on a variety of substrates. Results demonstrated that semen fluorescence is more intense than that exhibited by saliva, which can make saliva more difficult to detect. The weak intensity of the fluorescence exhibited by saliva can be attributed to the lack of solid particles within the saliva sample. Substrate type and colour had a significant effect on the detection of the biological fluid, with limited fluid detection on darker substrates. The porous nature of the white and dark blue cotton substrates meant the biological fluid was absorbed into the substrate rather than drying on the surface, leaving little surface fluorescence. Some substrates have inherent photo luminescent properties and can mask fluorescence from biological fluids, making them harder to detect. This technique acts solely as a screening method and can be used to inform and direct an investigator to the locations of biological staining during documentation of the scene. This technique cannot differentiate between biological fluids and any fluorescent areas will require further confirmatory testing to identify the fluid in question. In addition, where a fluorescent response is not observed, the presence of a biological fluid cannot be excluded. Further investigation is required to observe a broader range of substrates to determine the optimum conditions and limitations of this combined technique and its application for casework, particularly in the presence of alternative agents, which may also fluoresce, and therefore introduce false positive results. The unique real-time High Dynamic range (HDR) ability of the SceneCam significantly enhanced the detection of

biological fluids where background fluorescence masked target fluorescence. These preliminary results are presented as a proof of concept for combining 360^{o} photography using High Dynamic Range (HDR) and an alternate light source for the detection of biological stains, within a scene, in real time, whilst conveying spatial relationships of staining to other evidence. This technique presents the opportunity to rapidly screen a crime scene for biological fluids and will facilitate simultaneous location and visualisation of biological evidence.

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