Locating Semen on Live Skin Using Visible Fluorescence

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Abstract:

The location of semen on skin is very important in the clinical examination of sexual assault victims. Recent literature has questioned the effectiveness of Ultra Violet (UV) light to not only detect semen evidence but to distinguish such evidence from other material. This paper explores the effective use of visible fluorescence as an alternative to the UV fluorescence in locating semen on live skin. The effective use of fluorescence photography with both film and CCD cameras to capture hard copy images of semen are evaluated and discussed. The results show that effective location of semen on live skin can be achieved using visible fluorescence techniques.

1.0 Background:

In 1919 Dr Wood[1] published a paper in France and noted the potential use of Ultra Violet (UV) light for pathologists when examining human beings. He referred to a "black light". Over time this lamp and its medical application to humans has become termed as the "Wood's Lamp". Today there are several manufacturers who produce a Wood's Lamp, based on UV Radiation who's primarily use is the detection of semen on skin and in hair of victims of sexual assault.

The importance of examining skin and hair for semen in sexual assault cases is certainly of high importance. A 1981 paper by Enos and Beyer[2] relates eight cases where the only evidence to substantiate the allegations of sexual assault were obtained from skin and hair based on microscopic examination.

A recent paper by Santucci et. al[3] (Yale University School of Medicine) emphasizes the critical importance of detecting semen in rape cases, due to the newly emergent DNA sequencing technology. They conducted a detailed test of the Wood's Lamp with over 40 physicians and 29 semen samples. The object of this work was to determine the ability of these physicians to differentiate semen from other substances such as barrier creams and ointments. The conclusions were profoundly disappointing for the Wood's Lamp and in fact none of the physicians were able to differentiate semen and none of the semen samples were even found to fluoresce.

An abstract of this paper was then reported in the New Scientist in December 1999 under the heading "Missing Clues". This article reports that the traditional Wood's Lamp is in fact operating at the wrong wavelength and missing vital evidence. A British Police Surgeons representative is quoted as saying "I hope

the ones (Woods Lamp) we use are at the right frequency". Of course the reality is they are all exactly the same as those tested by Santucci.

1.1 Photoluminescence of Semen

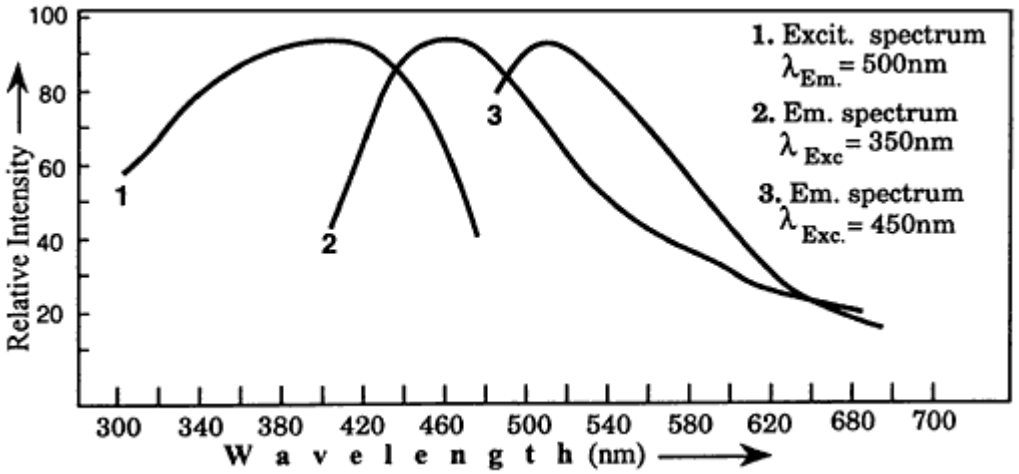

Figure 1. Excitation and emission spectra of dry semen on filter paper. Ref Stoilovic[4]

Figure 1 above (Milutin Stoilovic, AFP[4]) shows the absorption spectrum for dry semen on filter paper (ref trace 1). It shows a strong absorption between 300-450 nm. It also shows that if you illuminate dry semen with a band of light around 350 nm HPBW 40 nm (ie 330-370 nm), which is invisible to the human eye, then the semen will fluoresce, into the blue visible region (ref trace 2). The advantage of this is that you can make invisible semen stains appear visible to the naked eye.

Fig 1 shows there is an alternative to using UV when searching for semen stains. Illuminating dried semen with a band of light around 450 nm HPBW 40 nm (ie 430-470 nm) will produce strong orange fluorescence (ref trace 3) in a broad region with a maximum around 520 nm.

1.2 Visible fluorescence

The Woods Lamp technique is based on detection of semen by Ultra-Violet fluorescence. Invisible to the naked eye UVA radiation is used to excite the semen sample and produce fluorescence in the blue visible light region. In this case, only the induced fluorescence is visible to the naked eye. As discussed earlier however the background material may or may not produce its own fluorescence which may mask the semen fluorescence. The background material being perhaps cloth, hair or skin.

The Forensic Light Source technique is similar to the Woods Lamp, however, the technique used is visible fluorescence. The exciting light is visible to the naked eye and so the observer must wear special goggles to eliminate the bright excitation light and only let through relatively weak fluorescent light. In all cases the amount of fluorescent light produced is a small fraction of the excitation light.

The following schematic (figure 2) shows the visible fluorescence technique. In the visible fluorescence technique there are always two optical system to be considered, namely the excitation and barrier (emission) optical systems. The excitation filter must eliminate all of the potential light within the expected fluorescent region. It is essential that the blocking characteristics of the light source filters are greater than 10^{-6}. Otherwise light in the fluorescent region be incident on the sample background and will pass through the barrier filter, swamping the real fluorescence. Also the barrier filter must eliminate all of the excitation light, otherwise this light will also swamp the fluorescence.

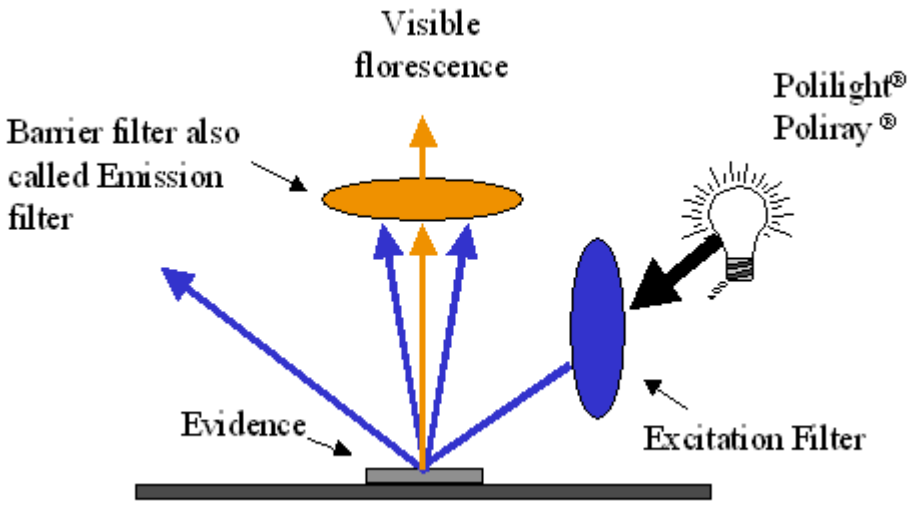

Figure 2. The visible fluorescence system requires two optical elements. One to produce the excitation illumination and a second to allow the visible fluorescence to be seen or captured.

Consequently in the visible fluorescence technique, a barrier filter needs to be used for either the human observer, or any recording device such as a film or CCD camera. In the case of the human eye, filter goggles are used and in the case of the film camera and CCD camera, camera filters are required.

As the amount of fluorescent light produced is always much less than the amount of excitation light, the quality both the excitation and barrier filter is of utmost importance. The most important feature of the filter is not its ability to pass light, but more it's ability to block the light it is designed to reject. The blocking ability of the filters (i.e. quality) is directly proportional to the sensitivity of the system to detect very small amounts of semen.

We also know from experience with weak fluorescent fingerprint evidence that CCD cameras with electronic integration provides instant viewing of long exposures which is not possible with standard film cameras. This system should provide better images for very weak or diluted semen stains.

1.3 Instrumentation

The instruments used in work reported here are the Poliray® and Polilight® forensic light sources manufactured by Rofin Australia Pty Ltd.

The Poliray® (ref fig 3 left) is a hand held forensic light source designed to be

highly portable. It uses a 75 Watt halogen lamp to produce white light. Highly selective interference band pass filters then produce the required visible light band of interest. The filters are tunable interference filters and are manually tuned. The light beam produced is focused to a even broad spot via a lens. The Poliray® is powered by either a battery or a transformer.

The Polilight® (ref fig 3 right) PL400 Bluescan<^{TM} forensic light source is a 400 W unit based on a special metal halide lamp. This unit produces UV as well as visible bands. The light is directed through a flexible two meter light guide that provides a highly concentrated and directional light beam. The light guide is liquid based for high energy transfer. The Bluescan^{TM} has an internal filter wheel driven by a stepper motor and can be controlled by either front panel buttons, a two meter hand held remote controller or optional PC controlled Windows® software.

Figure 3. The excitation systems used, (left) Poliray and (right) Polilight Bluescan^{TM}.

The barrier filter system used to capture the images in this paper is shown in figure 4 (right). The film camera has a barrier filter cassette system which is mounted onto the lens. The goggles shown in figure 4 (left) are high pass optical filters that have high blocking of the excitation light, and allow the operator to visualize the fluorescence.

Figure 4. The barrier filter system is goggles for direct viewing and a camera filter for photography.

2.0 Detection of Semen Using Visible fluorescence

2.1 Semen on Live Skin

2.1.1 Experiment

Semen samples from a single donor were collected and stored in a sterile container. After 12 hours semen was applied to the arm of a female volunteer and several fluorescent techniques were tried to visualize and photograph the semen while it was wet. In all cases no fluorescence could be detected.

Semen from the same donor was then re-applied and left to dry. In this case visualization through filter goggles and through the camera system were easily visible in a variety of fluorescence set-ups. The Poliray® and the Polilight® Bluescan™ in the illumination bands 415 nm +/- 20 nm, 450 nm +/- 45 nm, 470 nm +/- 20 nm and 490 nm +/- 20 nm provided the excitation light. The barrier filters used included high pass 475 nm, 515 nm, 550 nm and 590 nm and band pass filters 450 nm, 500 nm, 550 nm, 600 nm, 650 nm, 700 nm all with band widths of 40 nm and all of tunable interference type. (Interference filters are able to be down tuned 30 nm by tilting).

Whilst using the Poliray the semen could be visualized, photography on live skin was difficult due to the required exposure times which could be 10's of seconds. The extra power of the Polilight Bluescan™ made photography much easier on live skin.

Photography was taken in a dark room using a Nikon F100 camera with a Tamron 90 mm macro lens. Camera barrier filters system was as shown on figure 4. The film was Kodak 400 ASA colour negative film. Exposures were all F8 and varying in time between 0.5 - 12 seconds.

2.1.2 Results

Visualization of the dried semen stains through goggles and through the camera on live skin were successful in a large number of excitation and emission filter combinations. The best results in this single case were found to be obtained be using the Polilight Bluescan™ 415 nm +/- 40 nm band pass filter and a 475 high pass and 505 band pass +/- 40 nm interference filter. This combination provided the best visual contrast. Photography of this set up is shown below (note: click on the image for a larger image in a pop-up window.)

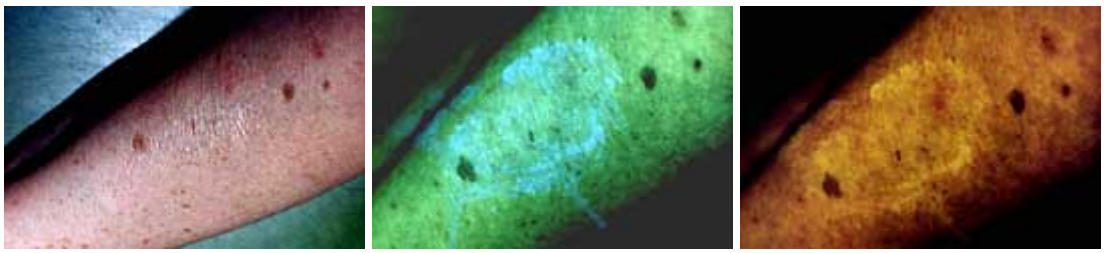

Figure 5. Left side shows female arm with dried semen photographed in daylight. The central photograph is taken with Bluescan™ at 415 nm and with a 475 nm high pass filter. The right hand photograph was taken with Bluescan™ at 450 nm and with a band pass 590 nm filter.

2.2 Semen in Hair

Recent work by Lincoln[5], McBride[5] and Turbett[5] investigated the use of forensic light sources (also termed alternative light sources) manufactured by Rofin Australia Pty Ltd.[6] for looking for trace evidence in sexual assault investigations. Lincoln et al conducted a survey into the ability of a light source to locate semen, in hair samples from a number of different donors. The following images are kindly provided by Dr Pam McBride (SARC[7]) and shows the type of results they produced during their investigation.

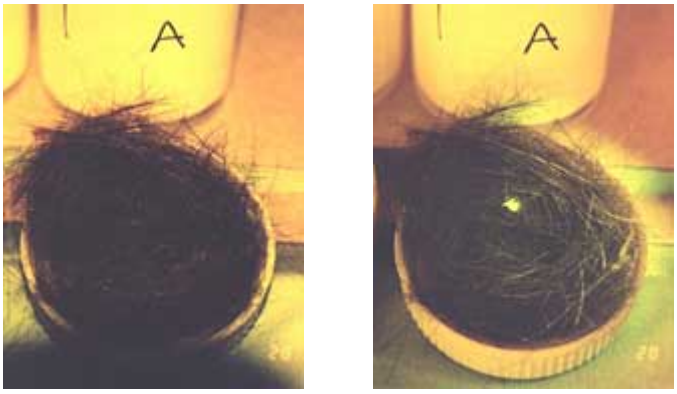

Figure 6. Left side shows a sample of head hair viewed through camera with 550 high pass (orange) filter and room light. Right side shows same sample but with Poliray™ blue light illumination, and orange high pass filter. The bright spot is semen.

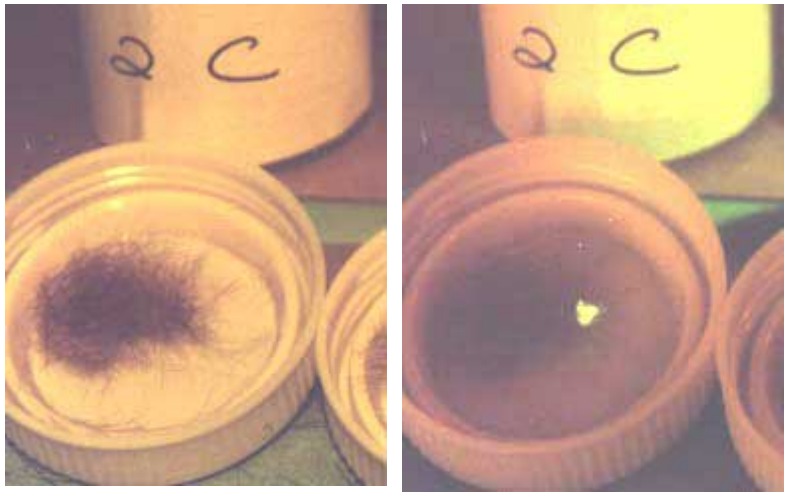

Figure 7. Left side shows a sample of head hair viewed through camera with 550 high pass (orange) filter and room light. Right side shows same sample but with Poliray™ blue light illumination, and orange high pass filter. The bright spot is semen.

As is evident in these images it is very obvious that semen is able to be detected. In these examples the fluorescent material identified was sampled and tested to confirm the material was indeed semen. In all cases the fluorescent material

was confirmed to be semen.

3.0 Conclusion and Comment

The work reported here is an initial investigation to determine the ability to locate and photograph semen on live skin and hair samples using visible fluorescence. It has been reported in the literature that the traditional Wood's Lamp was not able to successfully produce fluorescent semen samples from numerous donors. The Wood's Lamp uses Ultra Violet illumination with visible (blue) fluorescence.

Our results show that dried semen sample from a single donor, can be visualized under several visual fluorescence regimes on live skin. It is also obvious that fluorescent photography of these stains can be achieved. Further it is seen that semen samples in hair can also be successfully located and photographed (hair work authored and being reported by Lincoln[5].)

Further clinical work is required now to determine the ability to detect and photograph semen from a larger sample of semen donors and on numerous skin types. The development of the system to distinguish semen from other possible contaminates needs to be investigated as this has also been identified by Santucci[3] as a problem with the Wood's Lamp. We have identified a number of regimes under which we can achieve very good fluorescence in semen using visible fluorescence. This flexibility is seen to be important in the future work in isolating semen from other fluorescing contaminates. Oils for example tend to absorb strongly in the UV region and so selecting higher optical regimes will perhaps help increase our selectivity of semen over such contaminates.

The physics and optics involved here show us that the sensitivity of the system to detect semen samples is directly proportional to the power of the excitation energy and also quality, or purity of the excitation energy. Whilst semen will almost always fluoresce, the fluorescent light will be swamped if the purity of the excitation energy is not extremely high. Consequently the ability to quickly and easily scan a large region of skin for semen will depend both on the power and purity of the system.

It is understood from our investigation that the examination of sexually assaulted people is required to be performed under different and often difficult circumstances. For example adult females or males who are perhaps highly traumatized can communicate the nature and circumstances of the abuse involved. This contrasts with Pediatric examiners, examining young children who are perhaps unable to communicate and in which cases of abuse may have occurred a long time before examination, if at all.

The development of a comprehensive system to aid in the location of semen on live victims therefore requires some consideration that the examination may be carried out in very difficult circumstances where examination time is very limited and intrusion needs to be minimal. In other circumstances these factors can be less critical. Also the ability to easily capture fluorescent images for evidential reasons is deemed important.

We are working towards providing an integrated high power, optically pure, and image capturing system, to provide investigators with a sensitive and

effective tool.

References:

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6. Rofin Australia Pty Ltd. Unit 6/42-44 Garden Boulevard, Dingley Melbourne 3172. Rofin@rofin.com.au Mr Alex Bennett, Ms Sharon Marshall, Dr Hadrian Fraval.
7. Dr Cathy Lincoln, Dr Pam McBride, Sexual Assault Resource Centre, PO Box 842, SUBIACO, Perth, Western Australia. 6008
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