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TECHNICAL NOTE

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Improving the Effectiveness of Fluorescence for the Detection of Semen Stains on Fabrics

ABSTRACT: The paper describes the use of the Polilight, a light source based on a xenon arc lamp, to exploit the fluorescence properties of semen as an aid to searching fabrics for stains in sexual assault cases. The broad excitation spectrum of semen allows the fluorescence to be generated at a range of wavelengths. This permits the excitation and emission conditions to be selected that minimize interference from background fluorescence of the fabric and thereby optimizes the contrast between the fabric and the stain. A common method for the observation of fluorescence is the use of colored plastic goggles or filters. The paper shows that the detection of fluorescence from semen stains is significantly enhanced using appropriate interference filters.

KEYWORDS: forensic science, forensic biology, fluorescence, luminescence, semen stains, semen detection

In cases of sexual assault, examinations often involve the searching of items such as clothing and bedding for seminal stains. Common methods that have been employed are:

Visual—Items are examined for stains visible to the naked eye that can appear as a crusty white to faint yellow stain.
Physical—Areas of seminal stains on cloth can have a different texture from the rest of the material.
Chemical—Using reagent impregnated paper to map areas for the presence of acid phosphatase.
Fluorescence—The use of ultraviolet light to exploit the fluorescence properties of semen.

Searching items for seminal stains can be a tedious process, particularly for large items such as bedding. Fluorescence methods are therefore attractive as they provide a rapid, non-destructive way of screening large items and a large numbers of items. However, a negative result from a fluorescent examination cannot positively exclude the presence of semen, and other methods then need to be employed. Thus, if the efficiency of fluorescence detection of semen can be improved, its effectiveness as a rapid screening tool would be enhanced.

Stoilovic (1) showed that the excitation spectrum of semen was broad and that the fluorescence could be generated with wavelengths from 350 nm (UV) through to 500 nm (blue green). It is well recognized that white materials can show strong fluorescence under UV light due to the optical brighteners in the cloth and detergents. This background fluorescence can mask the semen stain. Therefore, the ability to produce fluorescence from semen at longer wavelengths can be an advantage. The Polilight (Rofin Australia) is a high-intensity light source based on a xenon arc lamp and utilizes a series of interference filters to provide a selection of illuminating wavelengths. The Polilight is therefore ideally suited to exploit the broad excitation spectrum of semen.

Over the past few years the "Polilight" has been used routinely in the authors' laboratory for the detection of dried semen stains in sexual assault cases. The technique is used as a screening method to target suitable areas for acid phosphatase testing and subsequent DNA analysis. However, during the course of normal casework it has been found that the fluorescence of semen stains can be difficult to detect on some types of cloth. The problems were principally associated with cloth that showed strong fluorescence itself, with cloth that was highly absorbent (e.g., fleecy material such as used in sweat tops and track pants) or with dark-colored fabrics.

This paper reports on studies that were done to investigate the cause of these problems and from this knowledge attempt to improve the detection of semen by fluorescence.

Experimental

Light Source

The light source used in this work was a Polilight model PL10 (Rofin Australia). The Polilight incorporates ten band pass interference filters that allow selection of wavelengths from the UV (350 nm) through to the red (600 nm). The interference filters produce sharp edges to the transmitted wavebands and therefore minimize the transmission of spurious light outside the selected waveband, which would interfere with the fluorescent emission.

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Observation of Fluorescence

Fluorescence is the emission of light caused by the excitation of molecules with light of a specific wavelength. The emitted fluorescence is always observed at a wavelength longer than the incident excitation light (Stokes Shift). The intensity of the emitted light is very weak relative to the intensity of the incident light, and therefore it is necessary to observe the fluorescence through a filter that will screen out the incident light. The Polilight kit includes pairs of different-colored plastic goggles (yellow, orange, and red) as a convenient means of observing the fluorescence. As the excitation wavelength is increased, the goggle selection would move towards the red. The Polilight kit also provides a number of band pass interference filters for the same purpose. Although less convenient to use than the goggles, they are more effective in separating the fluorescent emission from the reflected incident light and other competing light due to the sharp edges of the transmitted wavebands.

The filters are available with a range of peak transmission wavelengths and bandwidths. The filters used in this study had bandwidths of 30 to 50 nm.

Preparation of Semen Stains

The following range of fabrics was selected for the experiments based on the author's casework experience of problematic surfaces: white cotton, pink and dark green fleecy, and pink satin (polyester). About 0.5 mL of semen was deposited on the fabrics using a pipette.

Investigation of the Effect of Fabric Fluorescence

The semen stains were illuminated under the following filter settings on the Polilight: UV, 415 nm, 450 nm, 505 nm, and 530 nm. The fluorescence was observed using first the Polilight-colored goggles as follows: clear for UV, yellow for 415 nm and 450 nm, orange for 450 and 505 nm, and red for 530 nm. Second, the fluorescence was observed through interference filters as follows: 505 nm for 415 nm excitation, 530 nm for 450 and 505 nm excitation, 555 nm for 505 and 530 nm excitation, and 595 nm for 530 nm excitation.

Investigation of the Effect of Absorption of Fluorescent Material into the Fabric

A solution of Rhodamine 6G in ethanol was deposited on the fabrics in the same way as the semen. Rhodamine 6G is a strongly fluorescent dye, and the fluorescence can be observed using excitation light of 505 nm viewed through a 555 nm filter or orange goggles.

Neat semen was deposited on cotton, polyester, and fleecy fabrics and the rate of absorption and fluorescence observed. The dried semen stains were then diluted by adding four or five drops of water from a pipette to the stain. The fabric was then dried and the fluorescence observed.

Effect of Washing

Semen stains were prepared on white cotton, pink polyester (satin), and pink fleecy materials and allowed to dry for 24 h. The fabrics were then washed in detergent and examined under the Polilight and tested for acid phosphatase. A semen stain that had been stored at room temperature for six months was treated in a similar way.

New unwashed white cotton was washed, allowed to dry, and semen deposited on the cloth. The fluorescence of this stain was compared with that on new unwashed cotton.

Results and Discussion

Fluorescence of Fabrics

The white cotton, pink satin, and pink fleecy material all showed strong fluorescence under certain conditions, and this reduced the contrast between the semen and the background, making the stain difficult to see and in some instances masked the semen fluorescence completely. It was therefore important to choose the combination of excitation and emission conditions that maximized the contrast between the semen stain and the fabric.

The following points highlight the principal issues to emerge.

1. The colored goggles gave very poor results on these high background surfaces, and significant improvement was obtained when the fluorescence was observed through the interference filters. The more selective transmission characteristics of the interference filters are clearly demonstrated in Fig. 1, which shows how the background fluorescence of the pink satin dominates when viewed through the orange goggles while clear separation of the semen fluorescence is achieved through the 530 nm filter.
2. The results on the pink satin and white cotton clearly demonstrate the importance of using the optimum excitation and emission wavelengths on these high background fabrics. The fluorescence of white fabrics under UV light is well known, and therefore it is not surprising to see that the contrast between the semen stain and the background improves markedly as the excitation wavelength is increased from 415 to 505 nm, as shown in Fig. 2.
3. The fluorescence spectra for the white cotton and pink satin were measured, and the excitation and emission bands together with the optimum conditions for observing the semen stains are shown in Table 1. This shows clearly how the optimum conditions for observing the semen fluorescence have avoided those conducive to generating background fluorescence of the fabric. In practical casework, knowledge of the spectra of the fabric being examined will not be available. However, it is a relatively simple process to try different combinations of excitation and emission wavebands to select the optimum conditions.
4. The most general conditions suitable for the observation of semen fluorescence were the 450 nm setting on the Polilight and observation through the 530 nm band pass filter. Other useful Polilight/band pass filter combinations in order of value were 450/555 nm, 415/530 nm, 415/505 nm, and 505/555 nm.

Absorbency of Fabrics

The rhodamine solution was rapidly absorbed into the fleecy fabric when applied to the surface. The fluorescence ranged from very weak to not detectable. Given the strongly fluorescent properties of rhodamine, this result confirmed that detection of fluorescent material would be markedly inhibited by absorption into the fabric.

Typically, the high viscosity of semen results in the fluid re-

TABLE 1—*Excitation and Emission maxima for white cotton and pink satin and optimum conditions for observation of semen fluorescence.*

Fabric	Excitation, nm	Emission, nm	Semen Observation (Ex/Em) nm
White Cotton	340–410	440–470	505/555
Pink Satin	490–530	570–620	450/530

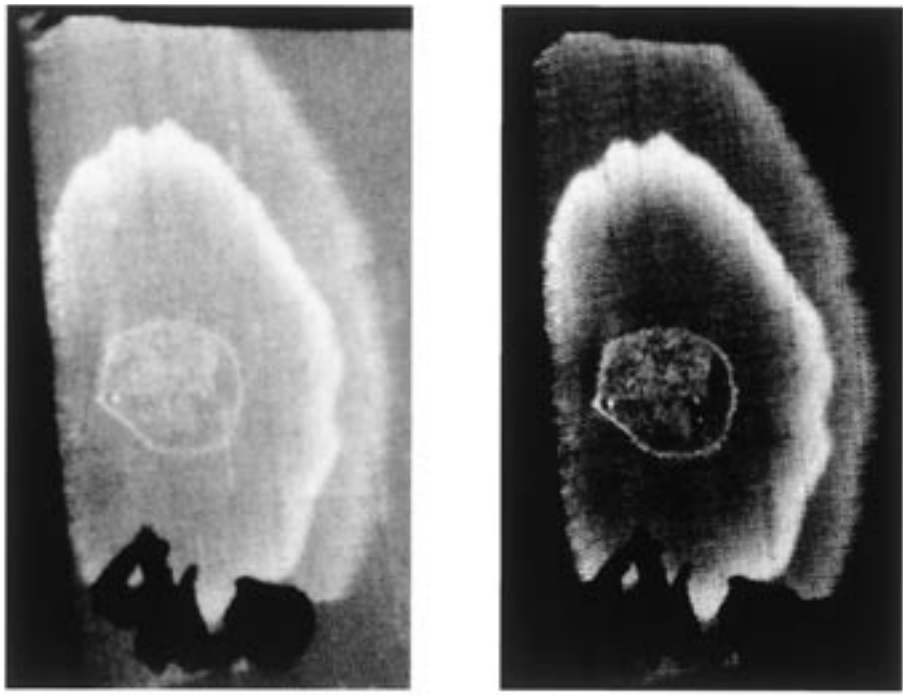

FIG. 1—*Semen stain on pink satin: (a) 450 nm excitation viewed through orange goggles—the background fluorescence of the pink satin has masked the stain; (b) 450 nm excitation viewed through 530 nm band pass interference filter—the more selective transmission characteristics of the interference filter provides a clear image of the stain.*

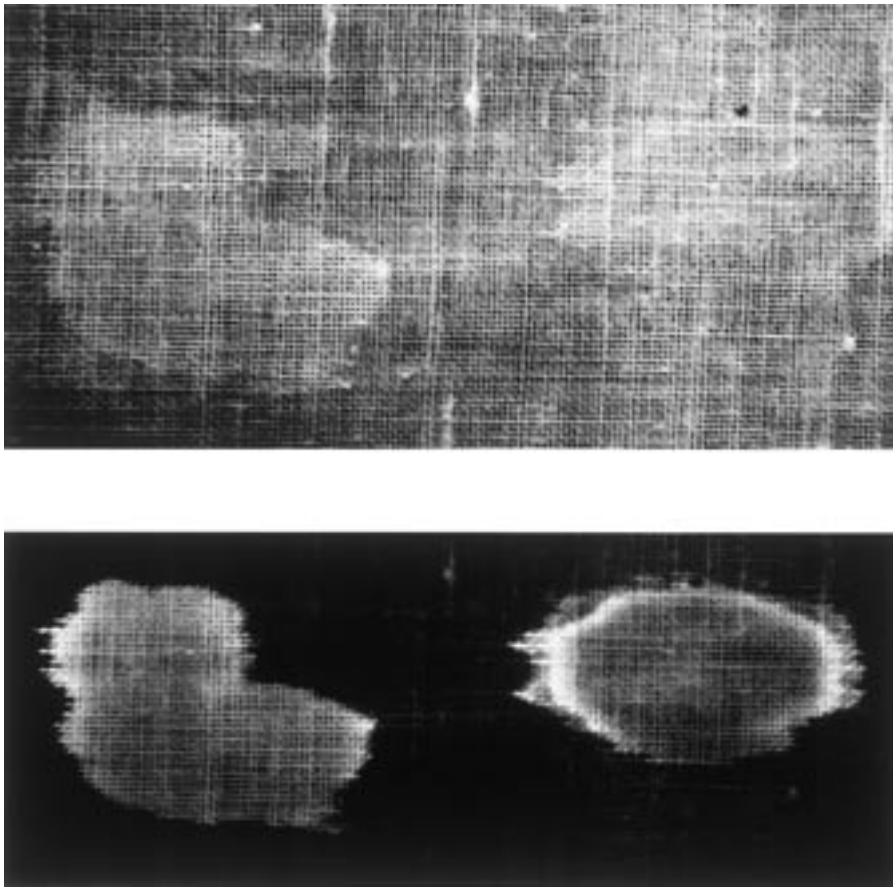

FIG. 2—*Semen stains on white cotton: (a) 415 nm excitation, viewed through 505 nm band pass interference filter. The strong background fluorescence of the fabric has obscured the stain under these conditions; (b) 450 nm excitation, viewed through 530 nm interference filter—the longer wavelength conditions have overcome the background, giving a clear image of the stain.*

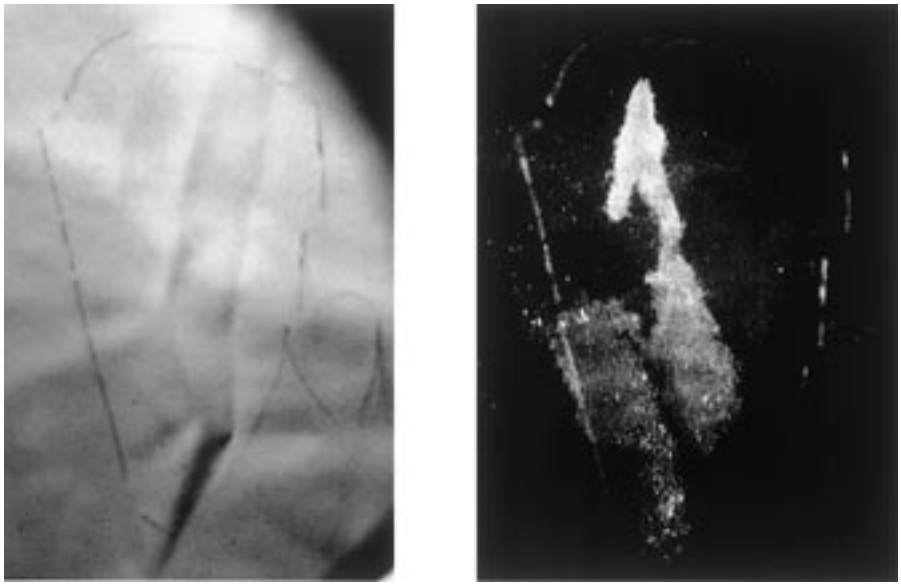

FIG. 3—*Casework example of semen stains on pink satin top: (a) 450 nm excitation viewed through orange goggles; (b) 450 nm excitation viewed through 530 nm interference filter.*

maining mainly on the surface when first applied to the fabric. While the wet semen remained on the surface of the fabric, it showed strong fluorescence. The rate at which the semen was absorbed varied depending on the type of fabric. In the case of the unwashed cottons and polyesters, absorption into the fabric occurred within 2 min, while with the washed fabrics the semen was rapidly absorbed within a few seconds. After the semen had been absorbed into the fabric, the fluorescence was initially greatly diminished. However, an interesting observation was that as the stain dried the intensity of the fluorescence increased.

In the case of the thick fleecy fabrics, virtually no absorption of neat semen occurred, and a dried stain that was strongly fluorescent was formed on the surface. However, dilution of the semen by the application of drops of water to the stain resulted in rapid absorption and permanent loss of fluorescence.

It is likely that with dark-colored materials the light-absorbing properties of the dye also affect the incident light and fluorescent emission if the semen becomes absorbed into the fabric.

The fluorescent semen stains often show the banded structure, as illustrated in Fig. 1. This suggests that there are multiple fluorescent components in the semen that experience a chromatographic separation as they migrate horizontally through the fabric. This process will, of course, also occur into the body of the cloth.

Effect of Washing

Weak fluorescence could still be detected in some stains after washing. The fluorescence of an old semen stain was stronger after washing than that of the fresh stains, suggesting that the aging process has made the fluorescent components of the semen more resistant to removal. The weakly fluorescent washed stains gave no response to the acid phosphatase test, suggesting that the fluorescent components are less easily removed.

The fluorescence of the semen stain on the pre-washed new cotton was significantly reduced in comparison to that on the unwashed material. This effect would appear to be due to removal of the surface treatment (seizing) on the cloth by the washing process, allowing the semen to be absorbed into the body of the fabric more easily.

Casework Examples

Example 1—A female victim was sexually assaulted and the offender ejaculated on the victim's breasts. The victim wore a patterned pink satin (polyester) top. No semen fluorescence was observed using the orange goggles. However, using 530 nm filter goggles and 450 nm incident light, extensive and detailed seminal staining was observed on several areas on the inner surface of the top as shown in Fig. 3.

Example 2—Following a sexual assault, the offender wiped his penis on a fleecy jumper. The jumper was then allegedly washed. Weak fluorescent staining was observed on the outer surface of the jumper. Sperm were recovered from these areas, which tested negative for seminal acid phosphatase.

Example 3—A male offender was observed masturbating under his towel at a public swimming pool. Before he was detained, he had swum in the pool. His bathers tested negative for seminal acid phosphatase; however, weak fluorescent stains were observed on the inner crotch surface. Sperm were recovered from these fluorescent areas.

Example 4—Routine screening of underpants worn by the victims of sexual assault has shown that blood, vaginal deposits, or faeces can mask seminal fluorescence. These materials simply cover the seminal stains and inhibit the fluorescence. In addition, vaginal drainage may dilute the semen, facilitating absorption into the fabric and a subsequent loss of fluorescence. Acid phosphatase testing to detect semen is strongly recommended in these cases.

Conclusion

The work has shown that there are two main problems to contend with when trying to observe the fluorescence of semen on fabrics: the inherent fluorescence of the material, and the effect of absorption of the semen into the bulk of the cloth.

The key to overcoming the fluorescence interference from the fabric is to exploit the broad excitation spectrum of semen (1). This allows a range of excitation and emission conditions to be tried in

order to optimize the contrast between the stain and the background. In addition, the excitation and emission bands need to have sharp edges. This is why the colored goggles were quite ineffective for observing the semen fluorescence on some fabrics compared with the interference filters. The authors have now had goggles purposely made with band pass interference filters as lenses for casework applications. The filters used in the goggles have a 40 nm bandwidth, and three sets have been obtained, covering wavebands centered at 505, 530, and 555 nm.

Fluorescence is significantly reduced or eliminated if the semen is absorbed into the bulk of the cloth and cannot be enhanced through varying excitation or emission conditions. This problem is particularly significant if the semen is diluted. In casework it has been noticed that semen deposited from vaginal drainage is more susceptible to absorption.

It is important to understand the advantages and limitations of fluorescent methods to maximize their effectiveness. Fluorescence cannot be used in isolation for semen detection but provides a valuable screening technique when used in conjunction with other methods. In the authors' laboratory, the sequence of examination is visible inspection followed by fluorescent examination to target areas for acid phosphatase testing.

Acknowledgments

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Reference

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