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# Methods and Applications in Fluorescence



## PAPER

### Analysis of the fluorescent properties of vaginal fluid upon ageing

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## Abstract

Detection and identification of body fluids are crucial aspects of forensic investigations, aiding in crime scene reconstructions and providing important leads. Although many methods have been developed for these purposes, no method is currently in use in the forensic field that allows rapid, non-contact detection and identification of vaginal fluids directly at the crime scene. The development of such technique is mainly challenged by the complex chemistry of the constituents, which can differ between donors and exhibits changes based on woman's menstrual cycle. The use of fluorescence spectroscopy has shown promise in this area for other biological fluids. Therefore, the aim of this study was to identify specific fluorescent signatures of vaginal fluid with fluorescence spectroscopy to allow on-site identification. Additionally, the fluorescent properties were monitored over time to gain insight in the temporal changes of the fluorescent spectra of vaginal fluid. The samples were excited at wavelengths ranging from 200 to 600 nm and the induced fluorescence emission was measured from 220 to 700 nm. Excitation and emission maps (EEMs) were constructed for eight donors at seven time points after donation. Four distinctive fluorescence peaks could be identified in the EEMs, indicating the presence of proteins, fluorescent oxidation products (FOX), and an unidentified component as the dominant contributors to the fluorescence. To further assess the fluorescence characteristics of vaginal fluid, the fluorescent signatures of protein and FOX were used to monitor protein and lipid oxidation reactions over time. The results of this study provide insights into the intrinsic fluorescent properties of vaginal fluid over time which could be used for the development of a detection and identification method for vaginal fluids. Furthermore, the observed changes in fluorescence signatures over time could be utilized to establish an accurate ageing model.

## 1. Introduction

Body fluid detection and identification are crucial aspects in forensic practice and can aid in selecting and prioritizing traces at a crime scene, as well as determining what activities might have taken place [1–3]. Tests for detection and identification should be sensitive, specific, rapid, easy to perform directly at crime scenes as well as non-destructive to enable subsequent DNA-analysis for donor identification [1]. Various techniques have been described for this purpose [1, 4–8].

Alternative light sources (ALS) are routinely used for the detection of biological traces at crime scenes

using the intrinsic fluorescent properties of the biological material [4, 5]. These methods are easy to use, non-destructive and applicable for detecting body fluids such as semen, saliva and urine on several substrates [9]. Large surfaces and surroundings can quickly be screened for visualization of biological traces using ALS. However, the ability to detect stains with this method is limited by the fact that fluorescence intensity and the optimal excitation and emission wavelengths can change when samples dry or upon ageing. Other limiting factors include the low-intensity fluorescence of some body fluids and the fact that some fluorescent- or highly absorbing substrates

can reduce or mask the stains' fluorescence [10, 11]. Additionally, ALS can only be used as an indicative test, but cannot be used to identify which body fluid is present [12].

To identify body fluids, presumptive tests are available, such as the Kastle-Meyer test to identify blood stains, the acid phosphatase test for semen identification and a test based on the enzymatic activity of alpha-amylase to identify saliva stains. In addition to presumptive tests, confirmatory tests are necessary to conclusively identify the traces [5]. A technique that has emerged as a promising new tool as confirmatory identification method is RNA analysis. Tissue-specific RNA expression can be used for body fluid identification, with a major advantage that RNA and DNA extraction can be performed simultaneously from the same stain [5]. However, a relative high amount of RNA must be present in the sample to allow tissue-specific RNA analysis, which limits the wider applicability of this method.

In sexual assault cases, vaginal fluid is frequently left as trace evidence. For example, on the suspect's fingers, clothing or contraceptives and can therefore aid in the identification of suspects or provide important evidence on what possible activities have taken place [13]. Despite the high forensic value of vaginal fluid, no rapid, non-destructive, on-site method is currently available for its detection and identification. The main challenges for such techniques are that the constituents of vaginal fluid 1) vary depending on the females menstrual cycle, and 2) differ largely among individuals due to differences in sexual activity, douching and chronic stress [14, 15].

Some destructive, presumptive tests are currently available for identification of vaginal fluid, such as the Periodic Acid-Schiff Reagent (PAS) test, examination of the ratio of lactic acid and citric acid or measuring the enzyme vaginal peptidase [4, 13]. In order to avoid destroying valuable DNA evidence, other studies have investigated non-destructive testing. A study performed by Virkler *et al* [6] used Raman microscopy to differentiate between blood, semen, saliva, sweat and vaginal fluid using characteristic Raman signatures obtained from the different body fluids. The spectra of vaginal fluid and semen contained comparable characteristic peaks, which was expected given that the body fluids share components like lysosome and urea. However, there were still some substantial differences. Several reference spectra were used to determine what components contribute to the Raman spectrum of vaginal fluid, but further research is needed. Sikirzhitskaya *et al* [7] also used Raman spectroscopy for the identification of semen, vaginal fluid and mixed samples. They were able to correctly identify all of the unknown samples, however only one-day-old stains were measured. Raman spectroscopy, despite its effectiveness, has limited sensitivity since it measures emission from both the body fluid and the substrate it is on. Substrates with even a low fluorescent background

interfere strongly with the Raman signals, hindering the identification of the target compound. Along with substrate interference, mixtures pose a challenge for Raman spectroscopy since the signal encompasses characteristics from all present substances, thereby complicating signal decomposition and identification [16].

Weber *et al* [17] examined the influence of the intrinsic fluorescence of vaginal fluid on the total fluorescence spectrum of menstrual blood. The peak intensity variability between peripheral and menstrual blood may indicate that vaginal fluid contributes to the fluorescence signal of menstrual blood. However, in their study, only three emission spectra were collected and the vaginal fluid stains were solely measured 2 h after deposition.

Ageing of body fluids possibly complicates the identification of body fluids since the fluid composition changes over time. However, these compositional changes can also be used to estimate the time of deposition of the body fluid. Bremmer *et al* [18] measured haemoglobin and its oxidation products in blood stains for age estimation with reflectance spectroscopy. In studies performed by Van Dam *et al* [19] and Achetib *et al* [3] protein and fluorescent oxidation products (FOX) in fingermarks and semen stains were measured with fluorescent spectroscopy. The formation of FOX occurs when samples composed of proteins and lipids are exposed to air. Specifically, unsaturated fatty acids will oxidize and form reactive oxidation products (LipOx). The LipOx will then react with the tryptophan-containing proteins within the sample, ultimately leading to the formation of FOX. They measured the relative quantities of protein and FOX over time to estimate the age of the traces [3, 19]. Although promising results were obtained with Raman spectroscopy for fresh vaginal fluids samples, the effect of ageing on the robustness of this technique remains unexplored [6, 7].

Therefore, the aim of this study is to examine the fluorescent characteristics of vaginal fluid over time with fluorescence spectroscopy to improve the detection and identification of vaginal fluid stains at the crime scene and gain insight in the temporal changes in the fluorescent pattern of vaginal fluid.

## 2. Material and methods

### 2.1. Participants and sample collection

Vaginal samples were obtained from healthy subjects who signed an informed consent. The medical ethics review committee of Amsterdam UMC reviewed the performed study and decided that the Medical Research Involving Human Subjects Act (WMO) does not apply to this specific study. Consequently, no specific study project number is available. Research protocols were performed in accordance with the Declaration of Helsinki and relevant guidelines and

regulations of the Netherlands Code of Conduct for Research Integrity and the research code of the Academic Medical Center. A total of 8 women participated in the study, of which 4 volunteers use contraception ( $32,5 \pm 9,3$  years) and 4 do not ( $31,3 \pm 6,8$  years). Samples were collected by instructing the participants to rotate a swab (4N6FLOQSwabs - Copan—4479429) within the vagina for 10 seconds. Donors were only included if they were not on their period and did not have sexual intercourse in the last 24 h. All samples were processed within 1 h after collection and stored in the  $-70$  °C freezer afterwards.

Processing of the samples was done by putting the tip of the swab in an Eppendorf tube together with  $50 \mu\text{l}$  of PBS (Dulbecco's PBS [1x] Capricorn Scientific - PBS-1A) and vortexing them for 15 min at 2850 rpm. An alcohol cleaned tweezer was used to take out the swab and place it in a DNA IQ spin basket (Promega-V1225). The basket was transferred to an Eppendorf tube and spun down for 1 min at 14000 rpm, after which supernatant was collected in a fresh tube. The supernatant was used for further analysis with fluorescence spectroscopy.

## 2.2. Sample preparation

For the fluorescence measurements,  $2 \mu\text{l}$  of sample for each time point: 0, 1, 7, 14, 21, 28, 56 days, was pipetted on a thin layer chromatography (TLC) silica gel 60 aluminium sheet (Merck – 105553) and left to dry for at least 30 min in the dark at room temperature. For each measurement a new spot was created to ensure that the long exposure time of (excitation) light did not affect the ageing process.

## 2.3. Excitation emission maps

Excitation and emission maps (EEMs) were measured with an LS55 luminescence spectrometer equipped with fibre optic accessory (Perkin Elmer, USC). The samples were excited at 81 different excitation wavelengths ranging from 200 to 600 nm with steps of 5 nm and a bandwidth of 10 nm. The emission was measured from 220 to 700 nm with steps of 0.5 nm with a detector slit of 7.5 nm. The speed was set to  $1500 \text{ nm min}^{-1}$ .

## 2.4. Ageing kinetics

Emission spectra in the wavelength range 313–400 nm (excitation at 285 nm) and 400–500 nm (excitation at 365 nm) were collected at each time point for every donor. The data for the spectra was obtained from the same measurements conducted for the EEMs. The autofluorescence intensities at these wavelengths are thought to be proportional to the relative protein and FOX concentrations, respectively [19, 20]. After calculating the area under the curve (AUC) of the protein and FOX related peaks the protein/FOX ratios were determined. With these ratios the ageing kinetics of the vaginal fluid samples could be monitored. To

determine whether there is a difference in ratio between the groups using contraception and without contraception, a paired T-test was conducted using GraphPad Prism version 9.3.1.

## 2.5. Data analysis

The same instrument settings were used for all the measurements of protein and FOX spectra at all the time points. Due to inter-sample differences, some samples exhibited relatively stronger fluorescence peaks than others at various time points. This saturated the detector of the spectrometer resulting in a flattened (clipped) peak which is much lower than what the 'real' peak would have been without the clipping. To reconstruct this 'real' peak, a cubic spline data interpolation algorithm was developed which interpolated between the non-saturated sides of the curve (Matlab software, Mathworks Inc., R2021a) (figure 1). To evaluate the peak correction method, samples that did not saturate were used as a test-set, the peak was manually clipped at a certain value and reconstructed using the algorithm. For each sample the absolute error between the AUC of the original and corrected graph was determined. The mean percentage error of all samples in the test-set was 0.27%, with a standard deviation of 0.20%.

Background correction was performed by measuring a blank TLC plate at each time point and subtracting this data from the obtained sample emission spectra. After the data corrections, the EEMS were normalized to allow for easy comparison.

## 3. Results

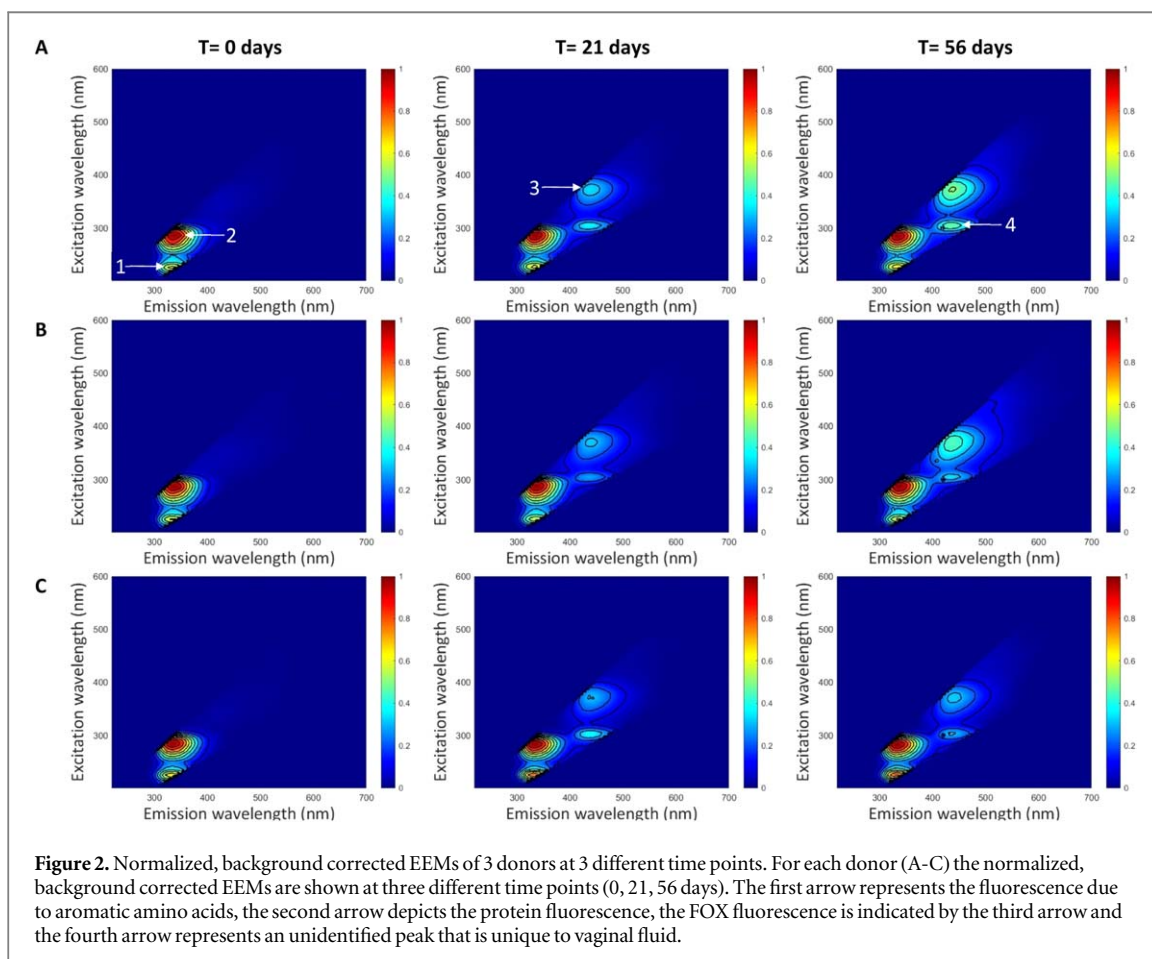
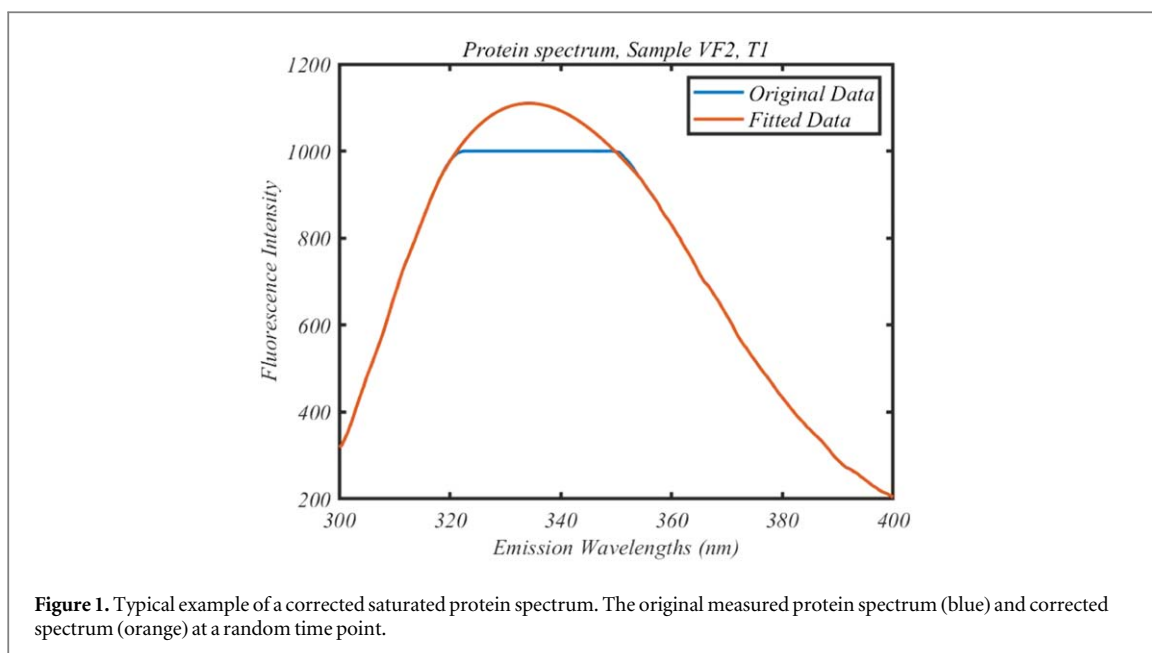
EEMs could be obtained from the vaginal fluid samples and illustrate distinctive fluorescence peaks at the different time points. To analyse the kinetics over time, the AUC of the protein and FOX related peaks were determined from the EEMs, after which the protein/FOX ratios were calculated.

### 3.1. EEMs

The normalized, background corrected EEMs of fresh vaginal fluid samples of all donors demonstrate two dominant emission peaks. One peak at 300–350 nm upon excitation at 220–230 nm and a second peak at 313–400 nm upon excitation of 285 nm (figure 2(A)). Over time, two other peaks were observed, namely around 400–500 nm when excited at 365 nm and around 425–450 nm at an excitation of 305 nm. The fluorescence intensity of the second peak decreases over time, while the intensity of the third and fourth peak increases upon ageing.

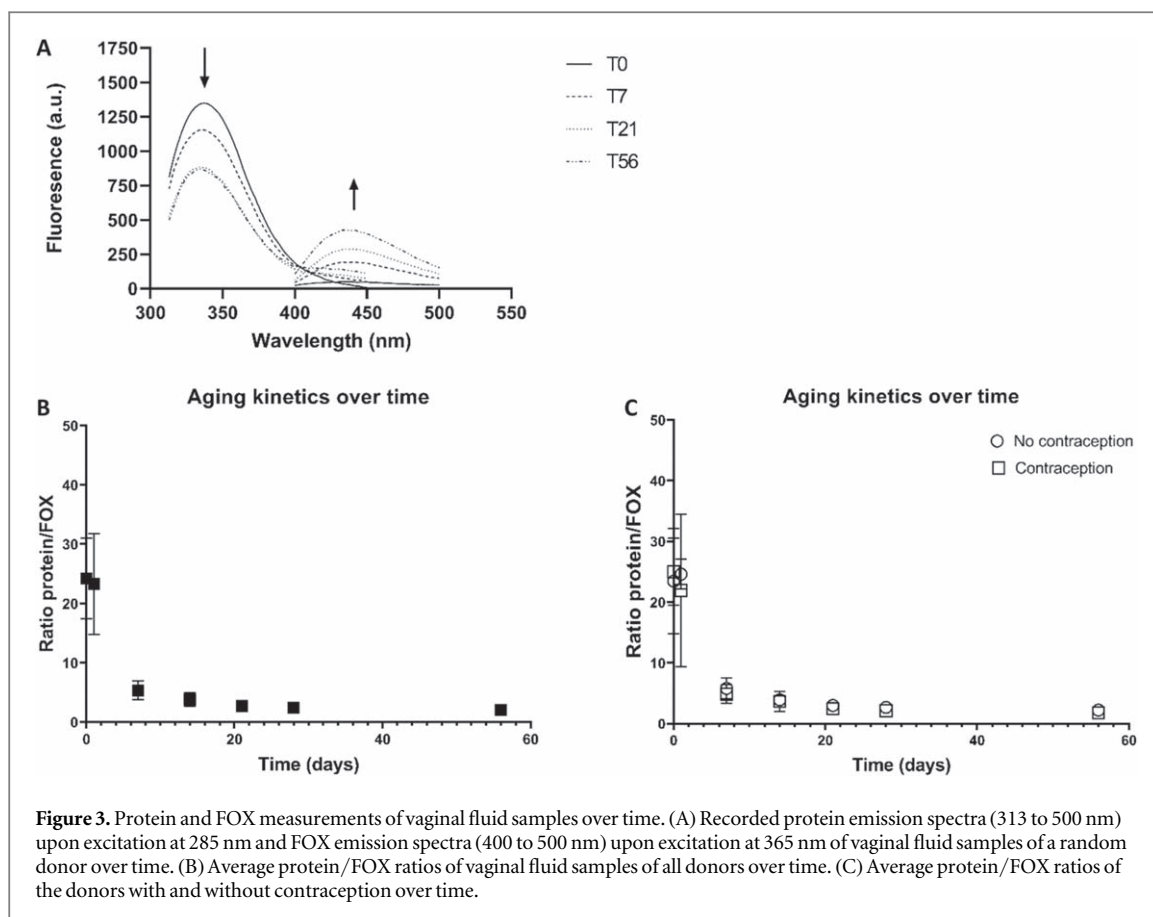
### 3.2. Protein/FOX ratios

To examine whether similar ageing kinetics could be observed in vaginal fluid samples compared to fingermarks and semen samples, the protein (excitation:



285 nm) and FOX (excitation: 365 nm) associated spectra were plotted. The protein peak decreased while the FOX peak increased over time, as illustrated by the arrows in figure 3(A), which is a typical example of the protein and FOX peaks upon ageing. The ratios of the AUC of protein and FOX over time were plotted to create an ageing kinetics curve. The mean protein/

FOX ratio over time is displayed in figure 3(B) for all 8 donors. To analyse if there is a difference in ratio between the groups with contraception and without contraception, the mean ratios for the two groups are shown individually in figure 3(C). The mean ratios of both groups follow a similar trend over time and no significant difference between the groups can be found



**Figure 3.** Protein and FOX measurements of vaginal fluid samples over time. (A) Recorded protein emission spectra (313 to 500 nm) upon excitation at 285 nm and FOX emission spectra (400 to 500 nm) upon excitation at 365 nm of vaginal fluid samples of a random donor over time. (B) Average protein/FOX ratios of vaginal fluid samples of all donors over time. (C) Average protein/FOX ratios of the donors with and without contraception over time.

( $p = 0.9204$ ). At time points T0 and T1, both groups have standard deviations greater than  $\pm 5.51$ , while from time point T7 the standard deviation decreases to less than  $\pm 1.71$ . This indicates that there is a large variety between fresh samples which diminishes over time. The relative error of each sample maintains consistent during the measurements, indicating that the system is stable over time.

To verify that 285 nm was the optimal excitation wavelength to measure the protein fluorescence, the fluorescence intensity when exciting at adjacent excitation wavelengths were compared. Overall, the highest fluorescence peak was observed upon excitation at 285 nm, with only 8 out of 56 stains having higher emission peaks when excited at 280 nm and 5 out of 56 samples resulting in higher emission peaks when excited at 290 nm (data not shown). The same approach was done for the measurements of the relative concentration of FOX with an excitation at 365 nm. The data from the first two time points was excluded since the FOX emission was too low during the initial time points. Higher fluorescence emission peaks for 27 out of the 40 stains were found when excited at 370 nm, with a higher prevalence upon ageing (data not shown).

#### 4. Discussion

There is a need in the forensic field to develop techniques that can not only detect and identify

vaginal fluid at crime scenes, but are also non-destructive to enable subsequent DNA analysis. Since the (bio) chemical components of vaginal fluid vary depending on the female menstrual cycle it is challenging to develop a method to detect and identify vaginal fluid. Therefore, the aim of this study was to explore the fluorescent properties of vaginal fluid over time with fluorescence spectroscopy. Our study results provide new information about the fluorescent behaviour of vaginal fluid, revealing a unique fluorescent pattern which so far has only been observed for vaginal fluid.

In general, the intrinsic fluorescence of both fresh and aged vaginal fluid samples allows for identification by displaying a unique fluorescent signal. In addition to the specific signal, many similarities between the fluorescent characteristics of vaginal fluid samples and other forensic relevant biological samples were found.

Multiple similarities were found between the fluorescent characteristics of vaginal fluid samples and other forensic relevant biological samples [3, 8, 11, 19].

The first fluorescence peak that could be observed in both vaginal fluid stains and other biological samples such as semen, saliva, fingerprints, serum and menstrual blood, has an emission peak at 300–350 nm upon excitation at 220–230 nm [11]. This fluorescence peak is formed in proteins containing aromatic amino acids, i.e. tryptophan, tyrosine and phenylalanine, and is caused by the excitation of higher excited electronic states of aromatic residues [20, 21].

Fingermarks, semen and saliva showed a second fluorescence peak that was also observed in the vaginal fluid samples. In studies conducted by Van Dam *et al* [19], Achetib *et al* [3] and Nanda *et al* [8], the auto-fluorescent properties of these biological samples were examined and showed that an emission peak around 350 nm could be observed upon excitation around 285 nm. The peak was attributed to the presence of the aromatic amino acid, tryptophan, in proteins in fingermarks, semen and saliva. A similar fluorescence peak was detected in vaginal fluid samples and could therefore also probably be explained by the presence of tryptophan in vaginal fluid [22].

Another fluorescence peak that was seen in vaginal fluid samples around emission 400–500 nm upon excitation at 365 nm after approximately 7 days, was also observed in fingermarks and semen stains [3, 19]. Assuming that the presence of FOX is associated with this peak and considering that proteins and fatty acids are also present in vaginal fluid, it is possible that same oxidation process took place in the vaginal fluid samples and caused this peak.

In addition to observing the protein and FOX related peaks in the EEMs, the relative concentrations of protein and FOX were determined over time by calculating the AUCs of the associated emission spectra. Based on previous studies, the excitation wavelengths for protein and FOX, 285 nm and 365 nm respectively, were selected [3, 8, 12, 20]. While 285 nm was found to be the optimal excitation wavelength for protein measurements in the majority of the stains, excitation at 370 nm resulted in higher emission fluorescence peaks for FOX measurements in over half of the vaginal fluid stains. In this study it was found that vaginal fluid exhibits a similar trend in protein/FOX ratio over time as seen in fingermarks and semen samples [3, 19]. However, further research is needed to investigate the influence of excitation at 370 nm on the protein/FOX ratios.

Being able to estimate the time of deposition is extremely valuable for the forensic field as it can help criminal investigators, evaluating the strength of testimonies and distinguish between crime related and unrelated stains. Additional studies are needed to confirm the comparable ageing kinetics of vaginal fluid as in fingermarks and semen and to examine whether a similar ageing function can be used for the age estimation of vaginal fluid samples.

Besides the similar peaks observed in the EEMs, an unique fluorescence peak was discovered in vaginal fluid, which has not been seen in other biological samples. The peak, observed from approximately 7 days, has an emission around 425–450 nm when excited at 305 nm. Acid phosphatase, lactic acid, citric acid, acetic acid, urea, pyridine, glycogenated epithelial cells and vaginal peptidases are some of the main substances found in vaginal fluid [6, 23]. These compounds may oxidize and produce fluorescent by-products. However, semen also contains components such as acid

phosphatase, lactic–and citric acid, urea, lactate and lysozyme which therefore might not explain the specific peak. The microbiome, on the other hand, is unique to vaginal fluid, raising the possibility that the fluorescence is caused by components or oxidation products created by the microbiome [22, 24].

To identify which compounds contribute to the fluorescence peak a mass spectrometry (MS) analysis or a fast-protein liquid chromatography (FPLC) analysis with the NGC medium-pressure liquid chromatography system (Bio-rad, USA) could be performed [25, 26]. Both techniques are used to separate, quantify and identify many compounds. If the compounds that contribute to the fluorescence appear to be vaginal fluid specific, the fluorescence peak might be beneficial for identifying vaginal fluid samples.

The donors that participated in this study were of various ages, used no– or different types of contraception and probably were in different stages of their menstrual cycle. However, they still displayed comparable increasing fluorescence intensities when excited at 305 nm and decreasing protein/FOX ratios over time. Therefore, it may be assumed that the menstrual cycle or individual differences do not influence these fluorescent changes, which aids with developing a method for the detection, identification and eventually even age estimation of vaginal fluid. In order to validate that the menstrual cycle and individual differences do not affect the changes in vaginal fluid over time, future studies should examine larger sample sets, measure additional time points and should be aware of the stage of the menstrual cycle, age and use of contraception. Furthermore, the characterization of vaginal fluid mixtures is necessary to allow development of methods based on vaginal fluorescent signatures [7].

In conclusion, the fluorescent properties found in this study are in agreement with previous findings that have been reported for semen and fingermarks. These similarities probably originate from protein fluorescence and the fluorescence of FOX. Moreover, comparable ageing kinetics have been observed in vaginal fluid samples as in fingermarks and semen. The unique emission peak at 425–450 nm, can be used as a body fluid-specific fluorescent signature to distinguish vaginal fluid from the other fluids. Together, these findings can contribute to the development of detection and identification methods of vaginal fluid samples for forensic purposes as well as a potential future ageing model for vaginal fluid.

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## Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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## References

- [1] Harbison S and Fleming R 2016 Forensic body fluid identification: state of the art *Res Reports Forensic Med Sci.* **6** 11–23
- [2] Stravers C S, Gool E L, van Leeuwen T G, Aalders M C G and van Dam A 2019 Multiplex body fluid identification using surface plasmon resonance imaging with principal component analysis *Sensors Actuators, B Chem.* **283** 355–62
- [3] Achetib N, Wilk L S, Schwarz J C V, Lambrechts S A G, Van Leeuwen T G and Aalders M C G 2019 Estimating the Time of Deposition of Semen Traces using Fluorescence Protein–Lipid Oxidation Signatures *Anal. Chem.* **91** 42
- [4] Virkler K and Lednev I K 2009 Analysis of body fluids for forensic purposes: From laboratory testing to non-destructive rapid confirmatory identification at a crime scene *Forensic Sci. Int.* **188** 1–17
- [5] An J H, Shin K-J, Yang W I and Lee H Y 2012 Body fluid identification in forensics *BMBRep.* **45** 206
- [6] Virkler K and Lednev I K 2008 Raman spectroscopy offers great potential for the nondestructive confirmatory identification of body fluids *Forensic Sci. Int.* **181** e1–5
- [7] Sikirzhyskaya A, Sikirzhyski V, Pérez-Almodóvar L and Lednev I K 2023 Raman spectroscopy for the identification of body fluid traces: Semen and vaginal fluid mixture *Forensic Chem.* **32** 100468
- [8] Nanda K D S, Ranganathan K, Umadevi K M and Joshua E 2011 A rapid and noninvasive method to detect dried saliva stains from human skin using fluorescent spectroscopy *J Oral Maxillofac Pathol.* **15** 22
- [9] Lee Wee Chuen K B E 2010 Forensic light sources for detection of biological evidences in Crime Scene Investigation: a review *Malaysian J Forensic Sci.* **1** 1
- [10] Miranda G E, Prado F B, Delwing F and Daruge E 2014 Analysis of the fluorescence of body fluids on different surfaces and times *Sci. Justice* **54** 427–31
- [11] Achetib N, Falkena K, Swayambhu M, Aalders M C G and Van Dam A 2023 Specific fluorescent signatures for body fluid identification using fluorescence spectroscopy *Sci. Rep.* **13** 3195
- [12] Aparna R and Iyer R S 2021 Detection and visualization of human tears using alternate light sources for forensic purposes *Sci. Justice* **61** 771–8
- [13] Igo A, Doi Y and Sakurada K 2015 Identification and evaluation of potential forensic marker proteins in vaginal fluid by liquid chromatography/mass spectrometry *Anal Bioanal Chem.* **407** 7135–44
- [14] Chen X, Lu Y, Chen T and Li R 2021 The Female Vaginal Microbiome in Health and Bacterial Vaginosis *Front Cell Infect Microbiol.* **11** 631972
- [15] Hickey R J, Zhou X, Pierson J D, Ravel J and Forney L J 2012 Understanding vaginal microbiome complexity from an ecological perspective *Transl Res.* **160** 267–82
- [16] McLaughlin G, Sikirzhyski V and Lednev I K 2013 Circumventing substrate interference in the Raman spectroscopic identification of blood stains *Forensic Sci. Int.* **231** 157–66
- [17] Weber A, Wójtowicz A and Lednev I K 2021 Post deposition aging of bloodstains probed by steady-state fluorescence spectroscopy *J. Photochem. Photobiol., B* **221** 112251
- [18] Bremmer R H, Nadort A, van Leeuwen T G, van Gemert M J C and Aalders M C G 2011 Age estimation of blood stains by hemoglobin derivative determination using reflectance spectroscopy *Forensic Sci. Int.* **206** 166–71
- [19] Van Dam A, Schwarz J C V, De Vos J, Siebes M, Sijen T and Van Leeuwen T G 2014 Oxidation monitoring by fluorescence spectroscopy reveals the age of fingermarks *Angew Chemie - Int Ed.* **53** 6272–5
- [20] Bortolotti A, Wong Y H, Korsholm S S, Bahring N H B, Bobone S and Tayyab S 2016 e.a On the purported 'backbone fluorescence' in protein three-dimensional fluorescence spectra *RSC Adv.* **6** 112870–6
- [21] Baldewijns S, Sillen M, Palmans I, Vandecruys P, Van Dijck P and Demuysers L 2021 The Role of fatty acid metabolites in vaginal health and disease: application to candidiasis *Front Microbiol* **12** 705779
- [22] Ziklo N, Vidgen M E, Taing K, Huston W M and Timms P 2018 Dysbiosis of the vaginal microbiota and higher vaginal kynurenine/tryptophan ratio reveals an association with chlamydia trachomatis genital infections *Front Cell Infect Microbiol* **8** 18:8:1
- [23] Duar R M, Lin X B, Zheng J, Martino M E, Grenier T and Pérez-Muñoz M E 2017 e.a Lifestyles in transition: evolution and natural history of the genus *Lactobacillus* *FEMS Microbiol. Rev.* **41** S27–48
- [24] Chen X, Lu Y, Chen T and Li R 2021 The female vaginal microbiome in health and bacterial vaginosis *Front Cell Infect Microbiol.* **11** 271
- [25] Domon B and Aebersold R 2006 Mass spectrometry and protein analysis *Science* **312** 212–7
- [26] Esteki M, Shahsavari Z and Simal-Gandara J 2019 Food identification by high performance liquid chromatography fingerprinting and mathematical processing *Food Res. Int.* **122** 303–17