https://doi.org/10.2352/ISSN.2169-4451.2017.32.416

A method for detecting the fluorescence-emission wavelength and visualization of biological traces

Yun Zou[1], Bei Jiang[2], Wenbin Liu[1], Xuejun Zhao[1], Nengbin Cai[3], Xiaochun Huang[3], Liuhu Cui[3], Xianfeng Chen[2]; [1]Shanghai Key Laboratory of Crime Scene Evidence, Shanghai Research Institute of Criminal Science and Technology, Shanghai 200083, China; [2]Department of Physics and Astronomy, Shanghai Jiao Tong University, Shanghai 200240, China; [3]Shanghai Key Laboratory of Crime Scene Evidence, Institute of Forensic Science, Shanghai Public Security Bureau, Shanghai 200083, China; Corresponding email: zouyun1984@163.com*

Abstract

The contribution of biological trace detection has been fundamental in forensic cases. This paper describes a method for the rapid and non-destructive method for latent biological trace detection on the surface of the non-porous substrates by laser reflection. The absorption and emission of the biological trace from the spectrophotometer and fluorophotometer are analyzed. Visualization of latent biological traces is realized by femtosecond laser and its parametric amplification system. Applicability of the ultraviolet (UV) from femtosecond laser to latent blood fingerprints detection has rarely been reported, especially in the range of multi-wavelengths laser. Results demonstrated that the latent blood fingerprints can be visualized through UV imaging system instruments.

Introduction

In forensic science, trace analysis of biology residues on non-porous surfaces is a critical aspect, they are an essential piece of forensic evidence. Biological traces provide valuable information to, for example, link a suspect to a crime. Several analytical techniques try to confirm the presence of biological traces by chemical reaction [1], hyperspectral methods [2-9], infrared spectromicroscopy [10-12], multiband Techniques [13], using laser [14-23], inherent luminescence [24] and chemical treatments [25-27]. Analysis of genomic extracted DNA seems to be an effective method for discriminating the source of biological traces to a specific individual. Body fluids such as blood, semen, saliva, urine and sweat all contain DNA evidence. However, DNA analysis is a costly and time-consuming process and does not provide immediate feedback to the forensic investigators. The laser light act as a simple screening technique in identifying stains of body fluids .

Laser technology in the application of forensic science has been studied since the late 70's, and has become one of the leading techniques for non-destructive fingerprint detection. Since the discovery of Menzel et al., in 1977, that latent fingermarks could be detected by argon-ion laser due to their inherent luminescence. With the development of laser techniques, laser exhibits properties of monochromaticity, coherence and directivity. Due to the limit of gain medium, intensity and the wavelength of laser, very little effect on the visualization of latent body fluids. The femtosecond laser has the short pulse, high intensity and ultrabroad bandwidth. Application of femtosecond laser to latent blood fingerprints detection has rarely been reported, especially in the range of multi-wavelengths laser.

Experimental

Sample preparation

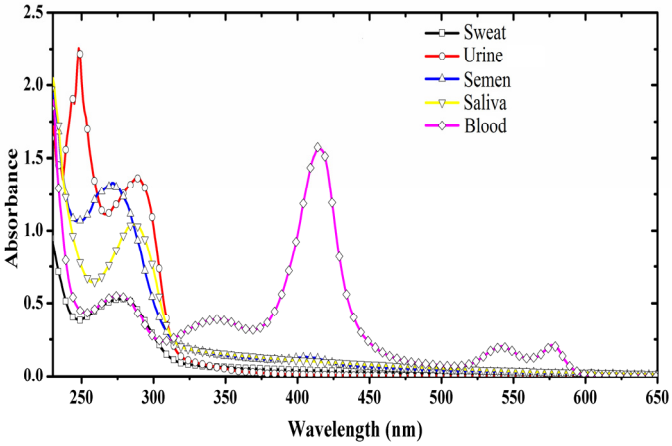
All experiments with human subjects were carried out in accordance with the guidelines of the National Institute of Health, Shanghai, China and approved by the Ethical Committee of Shanghai Jiao Tong University. All participants provided their written informed consent for the collection of biological samples. Five types of samples were obtained in liquid form from anonymous volunteers. Body fluids (blood, semen, saliva, sweat, urine) were diluted to 300 times with deionized water and injected into quartz cuvettes for testing.

Instruments

The absorption and emission wavelengths of body fluids were detected by spectrophotometer and fluorophotometer respectively. Femtosecond and its optical parametric amplifier were adopted for laser source. Images were recorded by CCD.

Results and discussion

Figure 1. *Absorbance spectra of body fluids (sweat, urine, semen, saliva, blood).*



The experiment demonstrated the absorption and emission wavelengths discrimination of body fluids. Fig. 1 illustrates the absorption characteristics of body fluids. The spectrum analysis is as follows: blood shows absorbance band at 250nm-300nm, 310nm-360nm, 370nm-450nm, 520nm-560nm and 570nm-590nm. Absorbance band of urine lies in the range of 266 nm-320nm.

Semen, sweat and saliva show absorbance band at 250nm-320nm, 250nm-320 nm and 260nm-320nm respectively. The absorbance spectra of body fluids are overlapped and mainly exists in ultraviolet (UV) band.

Fig. 2 shows the fluorescent emission bands of body fluids at different excitation wavelengths which are detected by fluorophotometer. The results shows that the strongest emission wavelengths of blood, semen, saliva, sweat and urine are 330nm, 345nm, 343nm, 350nm and 406nm respectively under the excitation of 280nm. When the excitation wavelength is set at 375nm, the corresponding emission wavelengths of blood, semen, saliva, sweat and urine are 485nm, 432nm-577nm, 430nm, 430nm and 446nm respectively. Emission wavelength of blood is 447nm under the excitation of 415nm. Strongest emission wavelengths of semen, saliva, sweat and urine are 577nm- 626nm, 460nm, 460nm and 515nm respectively under the excitation of 400nm. When the excitation wavelength is set at 530nm, the corresponding emission wavelengths of semen and urine are 575nm-629nm and 595nm.

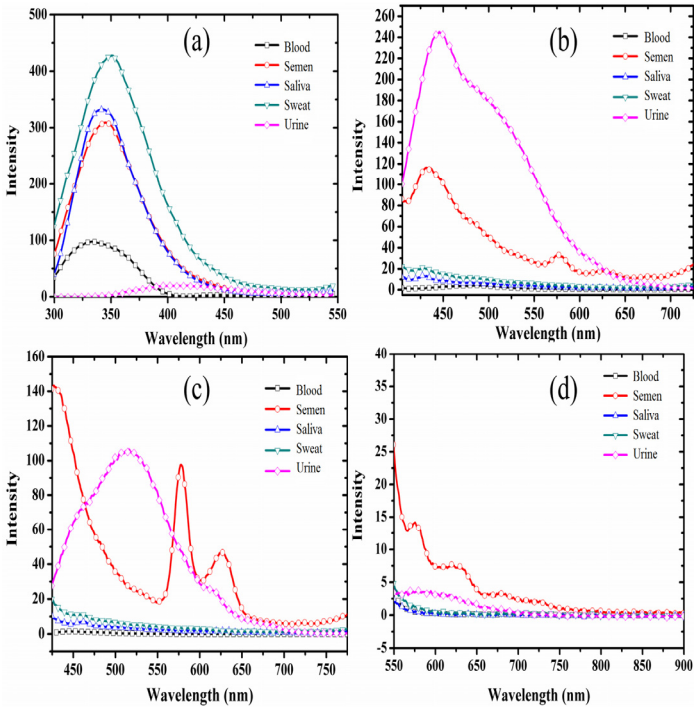


Figure 2. *Fluorescent emission bands of body fluids under excitation wavelengths of (a)280nm (b) 375nm (c)400-415nm (d)530nm*

Fig. 3 describes the layout of optical path for visualization of diluted blood stains with femtosecond laser at 415nm by reflection. In recent years the applied technology of UV reflection photography has shown great potential as a feasible technique for the enhancement of latent biological stains. The imaging of the system comprises of femtosecond regenerative amplifier system, optical parametic amplifier (OPA), prism, broad band filter (375-425nm), impervious objects, plate clamp, computer and ultraviolet CCD. A femtosecond laser controlled by the OPA was used. Measurements were taken using a laser emitting at absorbance peaks. CCD was set for acquiring images.

The initialized wavelength of femtosecond laser is 800nm, and it can be tuned to 415nm by optical parametric amplifier. Prism is introduced to eliminate the stray light from the laser. A laser beam incidents on the non-porous surface with diluted blood, and reflection light is received by UV-CCD for imaging.

Fig. 4 (A) and (C) shows the images under the natural light with diluted blood on slides and food package respectively. (B) and (D) presents the visualization images by UV laser.

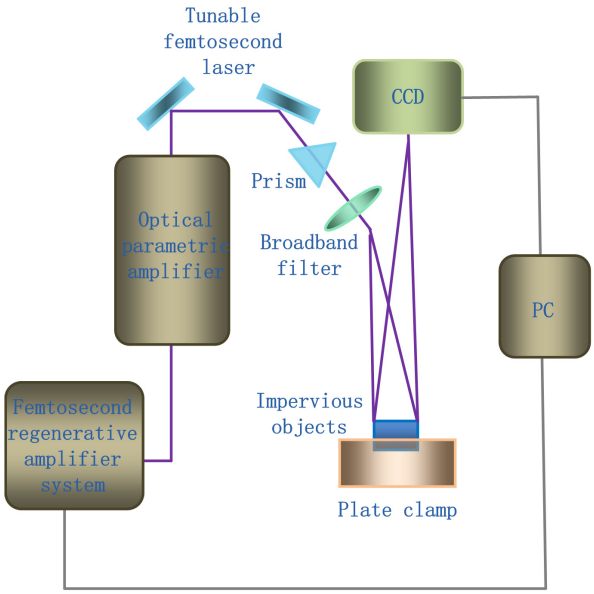


Figure 3. *The layout of optical path for visualization of diluted blood stains with femtosecond laser at 415nm by reflection*

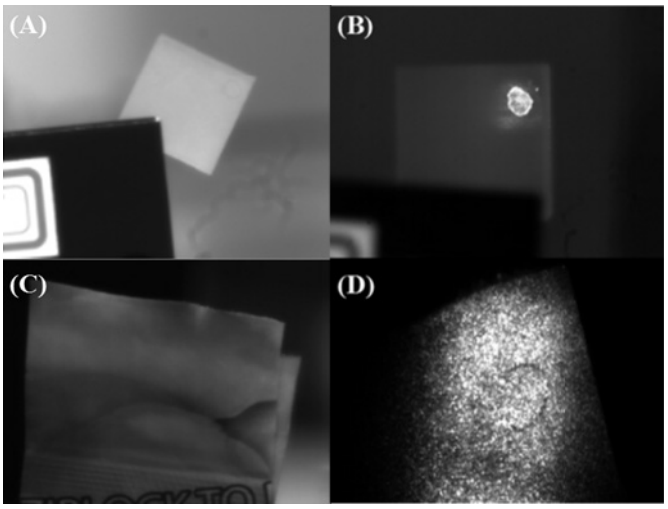


Figure 4. *(A) and (C) images under the natural light with diluted blood on slides and food package respectively. (B) and (D) presents the corresponding visualization images by UV laser.*

In our opinion, the extensive use of UV laser technology in the examination of latent evidence, in the laboratory or even at crime scenes, can help investigators obtain evidence and clues more quickly and accurately.

It is noteworthy that illuminating body fluid residues with shortwave UV radiation can have consequences in terms of affecting subsequent DNA typing results. It was found that treating latent stains with shortwave UV more than 5 min will precluded the acquisition of results from polymerase chain reaction (PCR) testing. PCR testing results can also be influenced by the power intensity and exposure dose from the shortwave UV laser.

Compared to chemical methods, this visualization technique is non-contact, free of reagents and interference from background. Recently, the application of visible absorption and

luminescence spectroscopic imaging studies on latent stains detection has produced promising results.

Conclusion

The absorption and emission of the biological trace from the spectrophotometer and fluorophotometer are analyzed. Visualization of latent biological traces is realized by femtosecond laser and its parametric amplification system. The Main advantage to using the laser or UV over chemical screening techniques is the fact that they are simple, nondestructive. The spectrofluorimetric analysis investigated in this study appears to be more utility than other techniques, which are based on fluorescence detection of body fluids. Diluted blood stains were detected with a high-intensity laser. UV illumination was used for detection of diluted blood stains invisible to the naked eye using femtosecond laser at 415nm by reflection. The spectroscopic method described has good sensitivity in detecting blood on non-porous surface.

Acknowledgement

We are grateful to Dr Xianfeng Chen, staff of Department of Physics and Astronomy in the Shanghai Jiao Tong University for the guidance on the spectrum analysis. This research was supported by the Project of National Natural Science Foundation of China (Grant No. 61405117), the Open Project of Shanghai Key Laboratory of Crime Scene Evidence (Grant No. 2014XCWZK03), the National Natural Science Foundation of China (Grant No. 6140031277), the Program of Science and Technology Commission Foundation of Shanghai (Grant No. 14JG0500300) and the Shanghai Science and Technology Development Funds (Grant No. 15QB1403400).

References

[1] D.L. Exline, C. Wallace, C. Roux, C. Lennard, M.P. Nelson, P.J. Treado, "Forensic applications of chemical imaging: latent fingerprint detection using visible absorption and luminescence," J. Forensic Sci. 48, 1047-1053 (2003).

[2] A.A. Gowen, C.P. O'Donnell, P.J. Cullen, S.E. Bell, "Recent applications of Chemical Imaging to pharmaceutical process monitoring and quality control," Eur. J. Pharm. Biopharm. 69, 10-22 (2008).

[3] A.A. Gowen, C.P. O'Donnell, P.J. Cullen, G. Downey, J.M. Frias, "Hyperspectral imaging – an emerging process analytical tool for food quality and safety control," Trends Food Sci.Technol. 18, 590-598 (2007).

[4] Levenson, R.M., E.S. Wachman, W. Niu, D.L. Farkas, "Spectral imaging in biomedicine: a selective overview, Proceedings of SPIE, Imaging Spectrometry IV," 3438, 300-312 (1998).

[5] G. J. Edelman, E. Gaston, T. G. Vanleeuwen, P. J. Cullen and M. C. G. Aalders, "Hyperspectral imaging for non-contact analysis of forensic traces," Forensic Sci. Intern. 223, 28-39 (2012).

[6] Bartick, E., R. Schwartz, R. Bhargava, M. Schaeberle, D. Fernandez, I. Levin, "Spectrochemical analysis and hyperspectral imaging of latent fingerprints," 16th Meeting of the International Association of Forensic Sciences, (2002).

[7] R. Bhargava, R. S. Perlman, D. C. Fernandez, I. W. Levin and E. G. Bartick, "Non-invasive detection of superimposed latent fingerprints and inter-ridge trace evidence by infrared spectroscopic imaging," Anal. Bioanalytical Chem. 394, 2069-2075 (2009).

[8] Suhre, D.R., L.H. Taylor, N.B. Singh, W.R. Rosch, "Comparison of acousto-optic tunable filters and acousto-optic dispersive filters for hyperspectral imaging," Proceedings of SPIE, 27th AIPR Workshop: Advances in Computer-Assisted Recognition, 3584, 142-147 (1999).

[9] M.D. Evans, C.N. Thai, J.C. Grant, "Development of a spectral imaging system based on a liquid crystal tunable filter," Transactions of the ASAE. 41, 1845-1852 (1998).

[10] N.J. Crane, E.G. Bartick, R.S. Perlman, S. Huffman, "Infrared spectroscopic imaging for noninvasive detection of latent fingerprints," J. Forensic Sci. 52, 48-53 (2007).

[11] A. Grant, T.J. Wilkinson, D.R. Holman, M.C. Martin, "Identification of recently handled materials by analysis of latent human fingerprints using infrared spectromicroscopy," Appl. Spectrosc. 59, 1182-1187 (2005).

[12] P.H. Ng, S. Walker, M. Tahtouh, B. Reedy, "Detection of illicit substances in fingerprints by infrared spectral imaging," Anal. Bioanal. Chem. 394, 2039-2048 (2009).

[13] L. J. Wang, D. Q. Tao, Z. Y. Yin and H. Zhao, "Multiband Techniques of LED and UV Materials on Visualization of Needle Marks," Adv. Mater. Res. 17, 1382-1386 (2012).

[14] B. E. Dalrymple, J. M. Duff and E. R. Menzel, "Inherent Fingerprint Luminescence - Detection By Laser," Proc. SPIE 0108, Optics in Security and Law Enforcement I, 118 (1977).

[15] B. Dalrymple and J. Almog, "Comparison of Latent Print Detection Using Semiconductor Laser and LED Light Sources with Three Chemical Reagents," J. Forensic Identification. 62, 14-27 (2012).

[16] N. B. Cai, X. C. Huang and S. W. Bo, "Study on the Development of Latent Fingerprints by UV Laser," Chin. J. Forensic Sci. 4, 20-22 (2010).

[17] A. Richards, "Reflected Ultraviolet Imaging for Forensics Applications," available on-line at http://www.ultravioletcameras.com/ (2010).

[18] P. Sanfilippo, A . Richards and H. Nichols, "Reflected Ultraviolet Digital Photography: The Part Someone Forgot to Mention," J. Forensic Identification. 60, 181-198 (2010).

[19] A. Richards and R. Leintz, "Forensic Reflected Ultraviolet Imaging," J. Forensic Identification 63, 46-69 (2013).

[20] N. Akiba, N. Saitoh, K. Kuroki, N. Igarashi, and K. Kurosawa, "Visualizing Latent Fingerprints on Color-Printed Papers Using Ultraviolet Fluorescence", J. Forensic Sci. 56, 754-759 (2011).

[21] A. P. Gibson, M. Bannister, and S. M. Bleay, "A Comparison of Three Ultraviolet Searching and Imaging Systems for the Recovery of Fingerprints", J. Forensic Identification. 62, 349-367 (2012).

[22] W. Sibbett, A. A. Lagatsky and C. T. A. Brown, "The development and application of femtosecond laser systems," Opt. Express 20, 6989-7001 (2012).

[23] E. Margalith, R.J. Barnes, "Spectral imaging device with tunable light source," I. Opotek, US7233392, 19-6-2007.

[24] BE Dalrymple, JM Duff, ER Menzel, "Inherent fingerprint luminescence-detection by laser," J Forensic Sci 22, 106-115 (1977).

[25] GF Wu, BS Liao, GG Yang, "Development of latent fingerprint using Nd: YAG laser," The Third National Meeting of forensic photography (China) 65-68 (1993).

[26] Z Yang, "The advantages of Nd:YAG laser on development latent fingerprint," Forensic Sci & Tech (China) 02, 41-42 (1995).

[27] YZ Zhang, F Jiang, "A robbery case cracked using laser excitation light," Forensic Sci & Tech (China) 197, 39-40 (2008).