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Enhancement of seminal stains using background correction algorithm with colour filters

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ABSTRACT

Evidence in crime scenes available in the form of biological stains which cannot be visualized during naked eye examination can be detected by imaging their fluorescence using a combination of excitation lights and suitable filters. These combinations selectively allow the passage of fluorescence light emitted from the targeted stains. However, interference from the fluorescence generated by many of the surface materials bearing the stains often renders it difficult to visualize the stains during forensic photography. This report describes the use of background correction algorithm (BCA) to enhance the visibility of seminal stain, a biological evidence that fluoresces. While earlier reports described the use of narrow band-pass filters for other fluorescing evidences, here, we utilize BCA to enhance images captured using commonly available colour filters, yellow, orange and red. Mean-based contrast adjustment was incorporated into BCA to adjust the background brightness for achieving similarity of images' background appearance, a crucial step for ensuring success while implementing BCA. Experiment results demonstrated the effectiveness of our proposed colour filters' approach using the improved BCA in enhancing the visibility of seminal stains in varying dilutions on selected surfaces.

1. Introduction

Appropriate visualization and detection of colourless biological stains such as seminal, saliva and urine at crime scene level is crucial for initiating further laboratory analysis to solve the crime [1]. Fluorescence imaging is a technique that enables visualization of untreated biological stains like semen [2,3], saliva and urine [3] as well as chemically treated trace evidences such as fingerprint and bloodstains [4]. Fluorescence is characterized by the absorption of light of shorter wavelength (excitation spectrum) by a substance and emission of light of a longer light wavelength (emission spectrum) [5].

Nevertheless, fluorescence (emission light) of seminal stains is considerably weaker compared to the excitation light. Therefore, light filters are often used to block the excitation light and permit only the seminal stains' emitted light during crime scene investigation [5]. For instance, fluorescence from seminal stains can be viewed through yellow or orange filters when illuminated by blue light. However, varying degree of fluorescence from the background materials can interfere with the fluorescence from the seminal stains rendering the latter less distinguishable. In this matter, a procedure for scanning the stained area using light of multiple wavelengths and filters was introduced [2]. Such approaches mainly used yellow, orange and red filters, which usually part of the forensic light source (FSL) and routinely available in crime scene investigation kits.

The advantages of using background reduction imaging techniques such as background correction algorithm (BCA) for enhancing visualization of diluted bloodstains has been reported [6,7]. BCA is effective in enhancing the visibility of diluted bloodstains, which have a strong narrow peak around 415 nm [2]. The effect of BCA in enhancing fluorescence from chemically treated fingermarks and bloodstains has also been studied [4]. This algorithm uses the spectral information from the stains when illuminated by light of two or three specific light wavelengths or when specified band-pass filters are used during photography. Wagner and Miskelly [6,7] utilized illuminations of varying light wavelengths such as 395 nm, 415 nm and 435 nm to perform BCA and reported that visibility of bloodstains was further improved after processing. The use of BCA can also improve the visibility of bloodstains using images illuminated with 415 nm and blue light (LED-based illuminations) that are commonly available in forensic light sources (FLS) [8].

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For transmittance/absorption mode, BCA [6,7] is calculated as

$$I_{out} = \frac{2I_1}{I_2 + I_3} \times 200 - 128 \quad (1)$$

where I_1 is the intensity of peak absorption and I_2 and I_3 are the light wavelengths of the two neighbouring images respectively. For instance, I_1, I_2 and I_3 are images of 415 nm (peak absorption), 395 nm and 435 nm (another two neighbouring wavelengths) respectively, as reported by Wagner and Miskelly [6,7]. Note that I_3 is substituted as I_2 for two-wavelength BCA, which resulted the calculation of the division part as I_1/I_2.

For fluorescence mode, BCA [4] is calculated as

$$I_{out} = I_1 - \frac{I_2 + I_3}{2} \quad (2)$$

where I_1, is the intensity of peak emission and I_2 and I_3 are the light wavelengths of the two neighbouring images, respectively. Note that I_3 is substituted as I_2 for two-wavelength BCA which resulting the calculation as $I_1 - I_2$. The output of this equation is adjusted to 0–255 manually for best viewing range.

The equations above do not include adjustment of brightness or exposure between images. In fact, this algorithm would fail if the brightness of image background differs. Therefore, it is crucial to ensure similarity in the brightness of the background among different images so that background in the resultant images would be as nearer to 1 and 0 as possible for transmittance and fluorescence, respectively. Note that best resultant images for transmittance mode would be stains appear dark and backgrounds appear bright, while it is the opposite for fluorescence mode where stains appear bright and backgrounds appear dark.

In this paper, we focus on the fluorescing property of seminal stains and the enhancement of seminal stains' visualization is performed through BCA in fluorescence mode (Equation (2)). Earlier reports have shown the successful use of BCA in improving the visibility of other fluorescing stains (none are about seminal stains) when using two or three narrow light wavelength band-pass filters. Furthermore, BCA has not been tested to discriminate fluorescing stain images captured using the commonly available colour filters in FLS kit.

Therefore, instead of using specific band-pass filters of narrow light wavelength, we implemented BCA on images captured with commonly used colour filters in forensic applications, namely yellow, orange and red. Two-wavelength BCA was utilized on two images of seminal stains: one captured with one of the aforementioned filters and the other captured without any filter. In fact, auto-exposure setting of camera could not ensure similar exposure or brightness for the images captured under the above two conditions. Therefore, we incorporated mean-based contrast adjustment (mCA) as pre-adjustment step on images prior to BCA, which was proven to be successful for enhancing different brightness images of bloodstains through absorption mode in our previous reported work [9]:

$$I_{out} = \frac{I_{in}}{I_{mean}} \times I_{target} \quad (3)$$

where I_{out} is the pixel intensity value of the output image, I_{in} is the pixel intensity value of the input image, I_{mean} is the mean intensity of the image and I_{target} is the brightness value of the targeted output that will be set as 1 in our algorithm.

The mCA is used to adjust the background brightness of images to be similar through division of their corresponding mean values. Therefore, our improved version of BCA, namely mean-based adaptive background correction algorithm (mABCA), is considered as more robust in processing images with background that differ in brightness. Compared to our previous two works for BCA [8,9], where both are reported for bloodstains through BCA in absorption mode (Equation (1)), we proposed novel approach for BCA using colour filters for seminal stains through mABCA in fluorescence mode in this study.

2. Theory

Fluorescent emission spectra of seminal stains have been shown to have light wavelength peak at around 450 nm and 510 nm under the excitation light wavelength ranges between 350 nm and 450 nm, respectively [2]. However, only three emission wavelengths were mentioned earlier [2]. In this research, we tested the spectral fluorescent characteristics of seminal stains using excitation light wavelength of wider range from 330 nm to 600 nm. Perkin Elmer LS55 Spectrofluorometer (PerkinElmer Inc.) was used to obtain the fluorescent spectra of seminal stains shown in Fig. 1.

It is seen that the emission spectrum from seminal stain has a significant peak remaining consistent at around 440 nm and that changes in the excitation light wavelength from 330 nm to 390 nm does not affect the position of this peak (Fig. 1a and b). Under excitation wavelengths of 415 nm and 430 nm, the peak itself was not observable but the patterns indicated strong fluorescence ending around 640 nm (Fig. 1b). On increasing the excitation wavelength to 450–510 nm, another emission peak was observed at around 510–560 nm (Fig. 1c and d), its peak position correspondingly increasing with an increment of 50 nm in the respective excitation wavelength. On further increment in the wavelength of excitation light, emission spectra without significant peak could still be observed (Fig. 1d and e) but their intensity becomes less significant, especially with excitation wavelength of 600 nm.

In this study, colour filters [Hoya Corp (supplied by Sirchie Inc.)], were used to filter the excitation light to allow wavelength at specific emission region to pass through during photography. The transmission curve of the colour filters reported in the specifications given [10] indicates the cut-on wavelength of yellow, orange and red colour filters to be around 480 nm, 540 nm and 600 nm, respectively. Therefore, seminal stains illuminated with 450 nm light wavelength can be viewed using yellow, orange or red filters to eliminate background reflection for rendering the stains observable. However, some backgrounds' fluorescence affected the fluorescence from seminal stains making it harder to recognize the latter even when observed through colour filters.

Correcting the background fluorescence using suitable algorithm would solve the above problem enabling better visualization of seminal stains. In this regard, two-wavelength mABCA in fluorescence mode was tested on images captured with and without filters. Since each of the red, green and blue channels of the camera has different sensitivity towards different lighting and filters, instead of selecting a single channel for mABCA, we converted the images into grayscale images by averaging the red, green and blue channels of each pixel. By integrating mCA (Equation (3)) into BCA for fluorescence mode (Equation (2)), our two-wavelength BCA equation becomes

$$I_{out} = \left(\frac{I_f}{\overline{I_f}} - \frac{I_n}{\overline{I_n}} \right) \times N \quad (4)$$

where I_{out} is the output image pixel intensity value, I_f is grayscale pixel intensity value of colour filtered images, I_n is grayscale pixel intensity value of non-filtered images, $\overline{I_i}$ is the mean intensity of their respective i image and N is the adjustment parameter. N is set as 255 to obtain the optimum range of 8 bit images, where all values larger than 255 were set at the maximum value of an 8 bit image, i.e. 255. This N value was selected after several trials of different values, where $N = 255$ gave output images with best