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Background Correction in Forensic Photography II. Photography of Blood Under Conditions of Non-Uniform Illumination or Variable Substrate Color—Practical Aspects and Limitations*

ABSTRACT: The combination of photographs taken at wavelengths at and bracketing the peak of a narrow absorbance band can lead to enhanced visualization of the substance causing the narrow absorbance band. This concept can be used to detect putative bloodstains by division of a linear photographic image taken at or near 415 nm with an image obtained by averaging linear photographs taken at or near 395 and 435 nm. Nonlinear images can also be background corrected by substituting subtraction for the division. This paper details experimental applications and limitations of this technique, including wavelength selection of the illuminant and at the camera. Characterization of a digital camera to be used in such a study is also detailed. Detection limits for blood using the three wavelength correction method under optimum conditions have been determined to be as low as 1 in 900 dilution, although on strongly patterned substrates blood diluted more than twenty-fold is difficult to detect. Use of only the 435 nm photograph to estimate the background in the 415 nm image lead to a twofold improvement in detection limit on unpatterned substrates compared with the three wavelength method with the particular camera and lighting system used, but it gave poorer background correction on patterned substrates.

KEYWORDS: forensic science, photography, blood, background correction

The concept of background correction in forensic photography has been developed and appropriate equations for both reflectance and transmission measurements given in a previous paper (1). This background correction is designed to enhance the detectability of a substance that has a narrow absorption band in the visible region compared with those typical of most substrates. The method is illustrated here by application to the detection of blood effectively using a two-dimensional extension of the Allen correction for blood quantitation (2). Thus, an estimate of the substrate reflectance at 415 nm (the position of maximum absorbance for fresh blood, and close to that for aged blood) is obtained by averaging images taken at 395 and 435 nm, and then this image is compared with the actual image taken at 415 nm. The most generally useful equations for detection of blood using this method are

$$\text{Corrected reflectance at } \lambda_2 \approx \frac{2\, I_{r,2}t_2}{I_{r,1}t_1 + I_{r,3}t_3} \qquad (1)$$

if the images obtained from the digital camera are "linear" and

$$\text{Corrected apparent absorbance at } \lambda_2 \approx \log(I_{r,2}t_2) - \frac{\log(I_{r,1}t_1) + \log(I_{r,3}t_3)}{2} \qquad (2)$$

if the images obtained from the camera are "non-linear" (the normal output from a consumer digital camera). In both these equations, $I_{r,i}$ is the intensity of light at wavelength λ_i detected by the digital camera, t_i is the exposure time used for that wavelength, and in the case of blood, wavelengths λ_1, λ_2, and λ_3 are 395, 415, and 435 nm, respectively. The "linear" images obtained from a digital camera are integrated intensity images so that $I_{r,2}t_2$ corresponds to the brightness of each pixel in a linear image. The non-linear image is approximately logarithmic, for example, $\log(I_{r,2}t_2)$ represents the intensity of each pixel in a nonlinear image taken at 415 nm. As noted in the previous paper, the background can also be corrected using only the 435 nm image (1). This is not as successful at reducing the effect of color differences on a substrate, but it has the twofold advantage of simpler image processing and removal of the need to take an image at 395 nm. (The light throughput in the alternative light source—digital camera system used in this study was significantly reduced at this wavelength, leading to the requirement for long exposure times.) This paper examines practical applications of this background correction technique. This includes methods for characterization and calibration of a consumer digital camera for application of this technique, as well as the limitations of using such a camera in this application. This material is included because digital photography in forensic science has usually been performed in a similar manner to traditional photography, rather than to the specifications required for other disciplines such as astronomy or military imaging (3). The limit of detection of blood on a range of substrates is also reported, together with methods for decreasing the limit of detection on selected substrates. Finally, artefacts that have been observed during development of this technique are discussed, together with their avoidance, reduction, or mitigation.

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Important Concepts and Camera Characterization

Pixels

The sensors in digital cameras are divided into pixels, each of which is a light detecting element. On most color digital cameras, each pixel detects only one channel of light, and the camera is designed so that there are twice as many green-sensitive pixels as red or blue to match the sensitivity of the human eye. There are alternative designs with multiple detectors or sequential acquisitions that can detect all colors at each pixel, but these are not found in most currently available consumer digital cameras. The number of pixels combined with the field of view determines the spatial resolution possible with a given digital camera. In our work we have used a Canon D30 camera, which has 2160 × 1440 active pixels, for a total of 3.11 M pixels. Thus, if our image is of an area of 18 cm × 12 cm, then each pixel represents an area of 83 μm × 83 μm, while if the area covered is 1.2 m × 0.8 m, then each pixel represents an area of 0.55 mm × 0.55 mm. However, since each pixel detects red or green or blue, the actual predicted spatial resolution is lower by a factor of two (for green) and four (for red and blue). Hardware and software anti-alias filtering degrades the resolution slightly further. However, in forensic applications, it is expected that the field of view will be chosen such that blood spots are not at the limit of resolution and that other factors will limit bloodstain visibility (vide infra). For this reason, camera characteristics other than the number of active pixels are likely to be important when evaluating a camera for use with the background correction technique described in this paper.

Bit Depth

Common consumer digital cameras can have bit depths of 8, 10, or 12. A bit depth of 8 means that the camera can report 256 levels of brightness for each of the three channels, so this depth is sometimes also referred to as 24-bit color. Higher-end consumer cameras can also provide 10 or 12-bit images, which have 1024 or 4096 levels of brightness in each color channel, respectively. These output levels still range in numerical value from 0 to 255, but fractional values are also allowed. Some scientific imaging devices allow 16-bit image capture, and image processing software such as Adobe Photoshop® can operate in 8-bit or 16-bit modes. Most data reported in this paper were exported from the camera in 12-bit mode, and then analyzed using Adobe Photoshop® in 16-bit mode. In many instances, blood can be detected using 8-bit images (although with less discrimination compared with the substrate), in which case lower-end image processing programs could be used.

Image Processing

Almost all consumer digital cameras acquire data using a sensor in which dyes positioned over pixels lead to sensitivity of individual pixels to red, green, or blue. Since each pixel only detects one color, the camera (or computer software) then needs to interpolate the colors so that the final image has a red, green, and blue component at each pixel. This interpolation process can lead to artefacts, especially at the edges of strongly colored objects, so most cameras or software also apply filtering to the interpolated image. Raw image files (see below) have not undergone this processing, but nearly all camera manufacturer software automatically performs some filtering on the raw file during its conversion to a viewable image.

Images obtained from a digital camera such as the Canon D30 can either be loaded into a computer as processed images (non-linear images) similar to that obtained from a film camera (often in jpeg format), or can be obtained as raw (linear) images in a 10, 12, or greater bit proprietary format. The raw images then need to be transformed into TIFF or other format images suitable for use in image processing software. Programs that can perform this transformation are provided by camera vendors, although third-party versions are also available. The transformation can either be linear (intensities recorded by the camera are converted directly) or approximately logarithmic (this corresponds more closely to the effects obtained with a film camera, and is referred to as "non-linear" in this paper). Aside from the color interpolation and filtering mentioned above, the linear transformation only allows for correction of the white balance of the image (i.e., the relative response of the red, green and blue channels—analogous to switching between daylight or tungsten film) which is primarily used to correct for different illumination sources in ordinary photography. Linear images look darker compared with "traditional" images, but they provide more intensity difference information over the range of brightnesses in an image. They are also more amenable to direct mathematical analysis since the non-linear images are not exactly logarithmic in light intensity.

Linear images obtained directly from the raw data do not necessarily have pixel brightnesses ranging from 0–255, since they are scaled directly from the sensor output. The linear images obtained from the Canon D30 camera used in this study showed saturated pixels (i.e., pixels having reached their maximum level) at a brightness level of about 160 (the exact value is dependent on the white-balance setting, the illumination source, and the color channel being observed). Non-linear images are processed such that the brightest pixels correspond to a level close to 255. In addition, non-linear processing often includes additional image processing such as alteration of overall contrast, saturation, and sharpness of the image.

Results

Characterization of the Digital Camera Images

Consumer digital cameras are designed to produce individual images that are visually pleasing. Where "photo-stitching" ability is offered, it is also necessary for sequential panned images of a given scene to be of similar brightness. However, when images are combined as discussed in this paper, the requirements on the camera are more stringent than those required by usual visual examinations. Therefore we report methods for determining the suitability and limitations of a digital camera for the background correction technique. Array size (number of active pixels) is not discussed explicitly, since most mid-level and higher consumer digital cameras currently available have greater than 2 million active pixels and with appropriate photographic technique images are therefore more likely to be limited by factors other than spatial resolution.

The repeatability of the camera was examined by photographing a grey scale card (Kodak) ten times at camera settings of linear mode, ISO 200, and shutter speed 1/350 s. For each of eight grey level patches on the card, a selection of pixels from the same region of each photograph was then selected, and the pixel intensities of this selection over all the ten photographs was analyzed, with the results shown in Table 1. A sufficient number of pixels (>1000) were used in the analysis so that interpixel variance had an insignificant effect on the estimate of the variance between images. Individual images had pixel noise ranging from a standard deviation of 0.6 at a mean pixel brightness level of 12 to 1.5 at a mean pixel brightness level of 120. (Saturation of the camera sensors led

TABLE 1—*Variability of photographs taken with Canon D30 (linear mode, 200 ISO, exposure time 1/350 s).*

Mean Brightness of Selection*	Within-image Standard Deviation of Selection†	Between-image Standard Deviation of Selection‡
169.73	0.56	0.14
121.39	1.53	0.82
85.01	1.23	0.59
58.51	1.04	0.41
39.28	0.84	0.28
26.75	0.76	0.17
17.62	0.68	0.14
11.95	0.59	0.09
5.69	0.44	§
3.61	0.34	§
0.12‖	0.11	§

* Each selection was a set of pixels of visually uniform brightness.

† Determined using the brightness filter in Fovea Pro on a selection of at least 1000 pixels.

‡ Determined from the mean brightness of identical selections in 10 separate images.

§ Not determined.

‖ Image taken with lens cap on camera.

to apparent lower variance at pixel brightness levels above 160 in linear mode.) In addition, there was between-photograph variation in overall brightness with a constant relative standard deviation of 0.7% at all brightness levels below 160. These results show that this camera has significant variability, both between pixels in a given uniform region of a photograph and also in the intensity of sequential photographs. Analysis of the data in Table 1 showed that the within-picture pixel variability depended on the average brightness level of the selected pixels, and that it could be fitted to an equation of the form:

$$\text{standard deviation of noise} = A + B^{*}\,(\text{mean pixel brightness})^{1/2} \qquad (3)$$

where A and B are constants for a given ISO value and exposure time. This suggests that there is both an intrinsic (constant) noise in the pixels and an additional contribution that is dependent on the integrated light intensity. This latter contribution is most likely due to shot noise, which follows a square root dependence on number of photons detected (vide infra) (4).

As noted above, the between-photograph comparison showed that the camera used had a variability in average brightness (as measured by the relative standard deviation between 10 images) of 0.7% between repeated photographs at an ISO setting of 200, Fig. 1. While this is barely observable when comparing two photographs visually, it could affect the use of photographic images in quantitative analysis. To ensure that the between-photograph variability was not due to the light source, a cloudless sky was photographed eight times in rapid succession (linear mode, ISO 200, 1/500 s) and then pixels in the same region of each photograph were analyzed. Again this indicated significant variability between images (relative standard deviation 0.9%). While the above results may suggest that this camera has no advantage over an 8-bit camera (in which the minimum difference between the 256 brightness levels is ±1) for pixel brightnesses above about 60, this neglects the noise reduction that can be achieved by averaging across pixels and the decrease in inter-image variability that can be obtained with the appropriate use of a grey scale standard. Furthermore, the 12-bit camera allows a greater dynamic range of brightness levels in a given image with dim pixels still containing significant information.

Characterization of the Within-Image Noise—the Photon Transfer Curve

The noise in a digital camera is typically due to three main types of sources: read and dark noise (constant, and most important at pixels exposed to little light), shot noise (equal to the square root of the number of photons causing the particular brightness level), and fixed pattern noise (due to different sensitivities of pixels in the image and so proportional to the brightness level). At very high pixel brightness levels, the noise appears to decrease, but this is an artefact due to pixel saturation (each pixel has a limited capacity) (4).

The dependence of the noise on brightness is often shown as a plot of log noise versus log mean pixel value, which is referred to as the photon transfer curve (4). Such curves for the Canon D30 used in this study at ISO settings of 200 and 400 are shown in Fig. 2*a*. The noise readings for these curves were obtained by photographing (ISO = 200 and 400; exposure times = 1/45 s and 1/180 s) a Kodak grey scale card twice, differencing the two images (while adding a constant offset to all the pixel levels in the differenced image) and then measuring the standard deviation of the pixel brightnesses in the differenced image over a uniform area corresponding to a given patch of grey. The standard deviation of the pixel brightnesses of a single image is the standard deviation so measured for the difference image divided by $\sqrt{2}$. The mean brightness values for the photon transfer curves were the mean values for each selected area from the original two photographs at each camera setting. This use of two images is designed to reduce the effect of fixed pattern noise so that the shot noise can be well characterized. However, we noted separately that determination of the noise of a single image gave a similar curve to that using two images, indicating that fixed pattern noise is not a significant source of noise for the camera and settings used for this part of the study. It should be noted that some digital cameras (including the Canon D30) have inbuilt routines that reduce the fixed pattern noise inherent in the sensor. At a shutter speed of 1/45 s and an ISO setting of 200 the mean value of a dark image (taken with the lens cap on) was 0.06 with a standard deviation of 0.05. Dark images at ISO 400 were slightly noisier, with mean values of 0.12 and standard deviation of 0.1 for exposure times less than 1/10 s. These values provide estimates of the combined dark current and read noise under these conditions. The linear regions of the photon transfer curves had slopes of 0.43 (ISO 200) and 0.40 (ISO 400), close to the value of 0.5 ex-

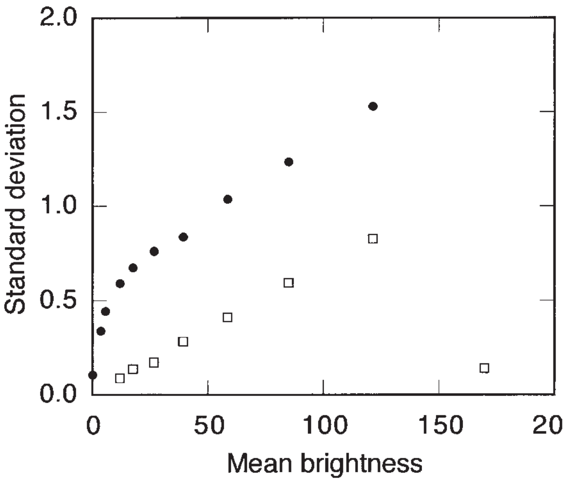

FIG. 1—*Pixel variability within a given photographic image (●) and between repeated images (□) as a function of mean pixel brightness.*

pected for shot noise. At high brightness values pixel saturation leads to deviations from the line (4). If the variance of the pixel brightness in the linear region of the photon transfer curve is plotted versus the mean brightness, a linear relationship is observed, Fig. 2*b*. The reciprocals of the slopes of these lines correspond to the number of photons detected per pixel that would lead to an increase of one brightness level. Since the camera used in this study appears to saturate at a pixel level of 160, this suggests it has a full well capacity for each pixel corresponding to ca. 39 000 at an ISO setting of 200. This capacity decreases to ca. 16 000 at ISO 400 since at higher ISO settings, the gain is increased so that each detected photon leads to an increased number of electrons in the pixel well. This is one of the sources of the increased noise at higher ISO settings. These full well values and dark noise readings can be combined to give the dynamic range of the camera (ca. 2700 at ISO 200 and ca. 1300 at ISO 400) (5). The combination of the dark and read noise is also increased at higher ISO settings as shown in Table 2. Furthermore, the dark signal is linearly proportional to the time of exposure. The Canon D30 has a special exposure setting to reduce this increased noise at long exposures by taking a second (dark) photograph of equal exposure time to the intended photograph and automatically subtracting the dark image. This corrects for systematic variations of dark signal between pixels, and the effect on the dark image is shown in Table 2. The successor to the D30, the Canon D60, apparently has lower dark noise so that this setting is not provided on the later model camera. The overall noise at any pixel brightness is determined by a combination of the read noise, the shot noise and, if it is significant, the fixed pattern noise. Since the shot noise is controlled by the number of photons corresponding to a given pixel value, this noise is going to be reduced by using as low an ISO setting as possible and using a camera with deep pixel wells (i.e., with a large number of detected photons corresponding to a full pixel well.) As noted above, the camera used in this study has noise of ca. ±1 at a mean pixel brightness of 100 when the ISO setting is 200. This noise will be a factor in determining the lower limit for the detection of low levels of blood unless pixel averaging (either by computer or visually) is used to decrease the interpixel noise.

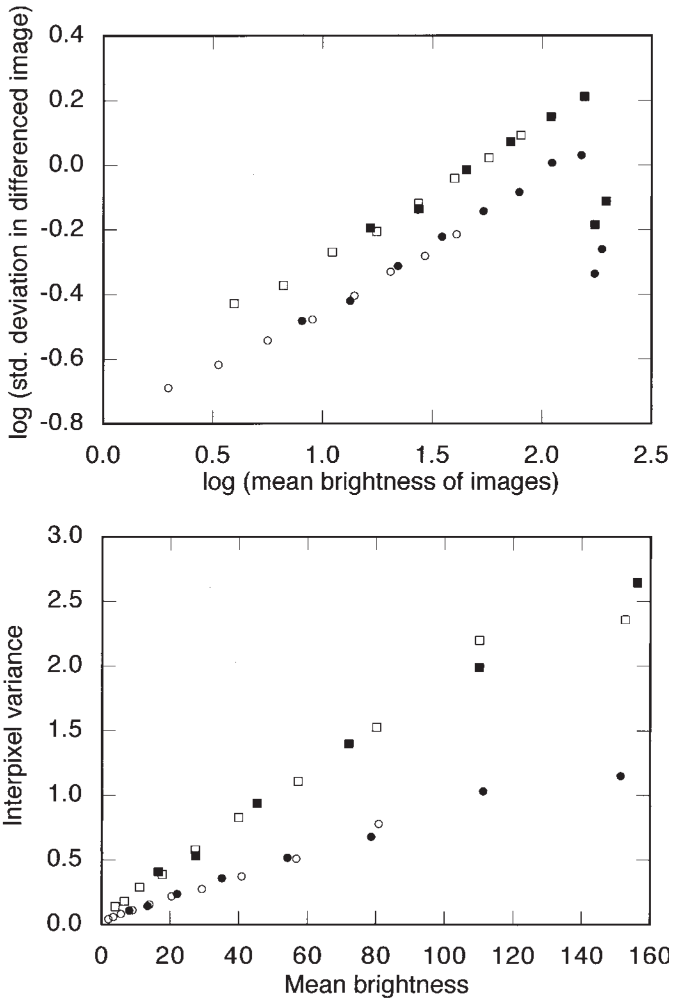

FIG. 2—a) *Photon transfer curves for the camera used in this study.* ○ *ISO 200, 1/180 s exposure time;* ● *ISO 200 1/45 s;* □ *ISO 400, 1/45 s;* ■ *ISO 400, 1/180 s.* b) *Graph of variance of pixels vs. mean pixel brightness for the camera used in this study. Symbols as in* a).

Noise Reduction in an Image

As noted above, the best method to reduce the noise in an image is to use an optimum combination of ISO setting and exposure time so that the regions of interest are well exposed while the ISO value is kept as low as possible. The further reduction of inter-pixel variability by an averaging process was investigated by applying a Gaussian filter with a radius of 1, 3, or 5 pixels in Adobe Photoshop. The Gaussian filter led to a decrease in pixel variation, as shown in Table 3, at the expense of some spatial blurring. As the line diagram in Fig. 3 shows, this reduction in interpixel variability should lead to an increase in detectability of bloodstains. However,

TABLE 2—*Effect of ISO setting and exposure time on dark noise for the Canon D30 camera used in this study. All images were obtained with the lens cap on the camera.*

	Exposure Times (s)					
	0.004	0.011	0.1	1	10	30
No noise reduction						
ISO						
100	0.055 (0.034)	0.048 (0.030)	0.057 (0.036)	0.060 (0.044)	0.37 (0.35)	0.98 (0.94)
400	0.13 (0.11)	0.12 (0.11)	0.13 (0.11)	0.17 (0.16)	1.1 (1.1)	2.6 (2.9)
1600	0.45 (0.46)	0.45 (0.46)	0.46 (0.46)	0.66 (0.65)	3.95 (4.50)	8.1 (11.3)
Noise reduction on						
ISO						
100	0.052	0.057	0.059	0.068	0.13	0.21
400	0.12	0.12	0.12	0.15	0.37	0.94
1600	0.51	0.39	0.41	0.64	1.5	7.2

Values are the mean values for areas of >2000 pixels. Values in parentheses are standard deviations of the selected pixel regions.

TABLE 3—*Effect of Gaussian smoothing (as implemented in Adobe Photoshop®) on observed noise at different pixel brightnesses.*

Mean Pixel Brightness	Gaussian Radius			
	None	1 Pixel	3 Pixels	5 Pixels
54.8	0.47*	0.42	0.20	0.14
28.3	0.32	0.22	0.20	0.16
15.0	0.20	0.13	0.065	0.075
8.6	0.16	0.11	0.10	0.047
5.6	0.13	0.12	0.069	0.031
3.8	0.11	0.06	0.027	0.016

* Values are standard deviations of pixels in 50–60 pixel segments of line profiles traversing images of a Kodak grey card. A single photograph was processed to obtain the four images.

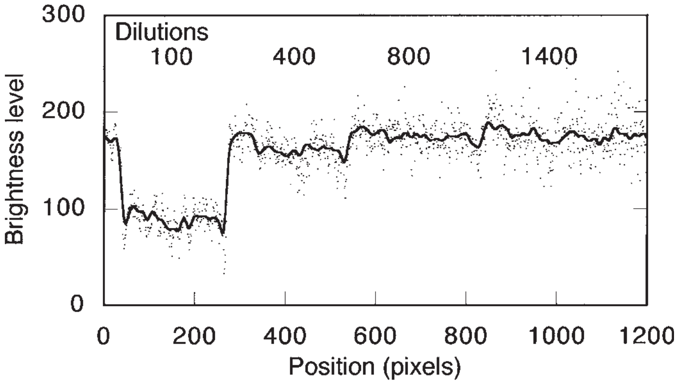

FIG. 3—*Effect of Gaussian blur on line profile through bloodstains of dilution 100, 400, 800, and 1400 on bleached cotton. The contrast of the background corrected image was optimised prior to the Gaussian blur. Dots are from unblurred image while line is for the same image with a Gaussian blur of radius 5 pixels applied.*

in practice, the visual detection of bloodstains that were reasonably large in comparison with the pixel size (test images often had blood spots with diameters of >50 pixels) by visual examination of processed images was not greatly affected by digital averaging because the human visual system also performs spatial averaging (6). This is discussed further in the section on detection limits (vide infra). Digital averaging can be useful if the image then has further contrast adjustment or is thresholded to delineate bloodstains (7). Other filters are available for when the noise is severely non-Gaussian or is periodic (7).

Linearity of the Camera Response

The linearity of the camera was then examined using the Kodak grey card. As can be seen in Fig. 4*a* the response in a given image was linear over at least the range 6 to 50. If linear mode photographs at a range of exposures (exposure times 1/500 s to 3 s) were compared the camera showed linearity over the total range of pixel brightnesses 1.5 to 160, indicating that it should be possible to apply the background correction equivalent to Eq 1 with the camera used in this study as long as the linear images taken at all three wavelengths have intensities in the region of interest between 1.5 and 160. This would only be true in the absence of noise; the presence of noise decreases this range, with an average pixel brightness of 6 being closer to the usable minimum for our system. Furthermore, studies over a range of conditions showed that deviations in linearity could sometimes occur at brightness levels above 140, so that this was used as the practical upper level. The linearity of the camera combined with the validity of Eq 1 was also examined. Thus, the photography (in transmission mode) of sequential dilutions of blood indicates that the illumination and camera system had a linear response to blood over at least a 40-fold change in blood concentration (1 mm deep solutions of 25× to 400× diluted bovine blood) using the three wavelength background correction technique discussed in this paper.

Exposure Limits

The optimal exposure settings for the three wavelengths background correction technique are approximately such that the substrate is exposed as if it is an 18% grey or one to two stops more exposed than this setting. This is most readily achieved if the item of interest is photographed against a backdrop of similar overall reflectivity, and if the camera is set on auto exposure. However, sometimes (especially for the 395 nm image) the camera we tested under-exposed images on the automatic exposure setting, in which case the exposure time for the 395 nm image was set at ten times that used for the 415 nm image (the factor will change depending on the illuminant and camera used). With the system used in this study (Polilight + camera), the light throughput is such that the exposure times at constant aperture setting are in the approximate

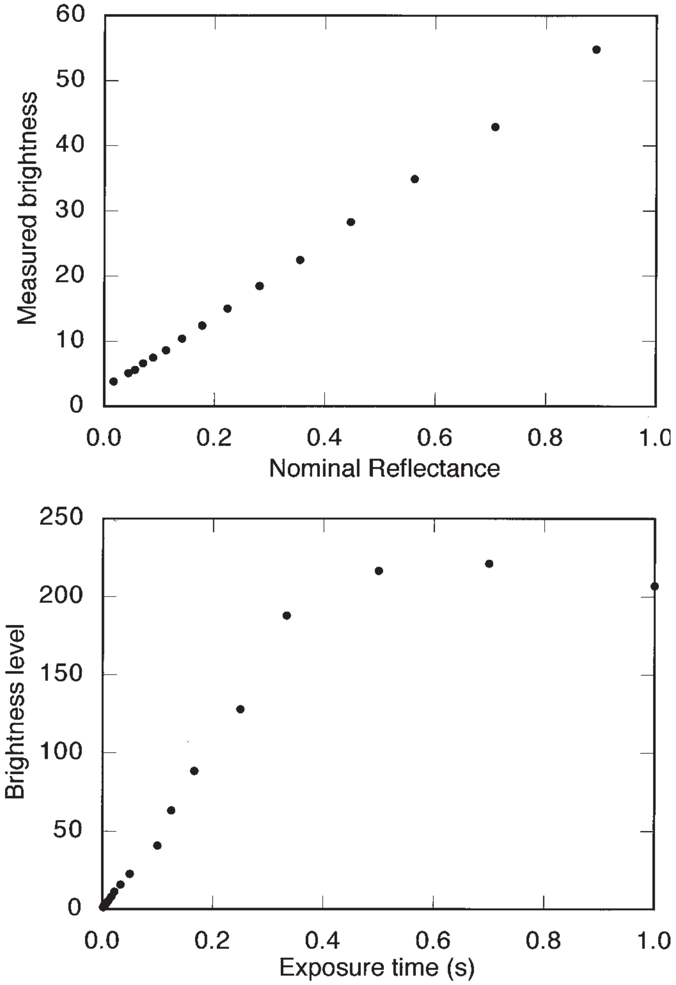

FIG. 4—*Tests for linearity for the camera used in this study.* a) *Linearity test based on a single image of a Kodak grey scale card.* b) *Linearity test based on images obtained with variable exposure times.*

ratio 1: 10: 100 for 435, 415, and 395 nm, reflecting both the drop in light intensity from the Polilight and the decrease in camera sensitivity. The exposure problems at 395 nm are most likely due to the room used for large items not being completely lightproof, so that stray non-monochromatic light was affecting the auto-exposure function on the camera.

The three wavelength processing described in this and the previous paper (1) only works when pixels in the images at all three wavelengths are in the linear range of the camera and under conditions where noise is not significant compared with the image intensity. This means that the pixels in the region of interest cannot be near saturation, and that in addition areas cannot be underexposed. In practice for our camera this leads to the requirement that in linear images the pixels have to have brightness levels less than about 140 and above about 6. This does limit the overall dynamic range of photographs suitable for processing using this technique; however, exposure bracketing can overcome this issue.

If images do have saturated or too dark pixels, processing according to Eq 1 no longer gives accurate representation of excess absorbance at 415 nm, and instead light or dark artefacts can appear in the processed image. It is possible to mask these areas of incorrect exposure using the threshold function in a program such as Adobe Photoshop® to avoid false identification of potential blood spots. An example of the artefacts caused by pixel saturation is shown in Fig. 5*b*. These artefacts were due to the 415 nm and 435 nm images being overexposed (pixels were being saturated). Thresholding the 435 nm images at a pixel brightness level of 140 led to the mask shown in Fig. 5*c*, which clearly matches the artefacts in the image. Combination of the mask with the processed photograph removes the false positive stains, Fig. 5*d*. Figure 5*e* shows the processed image obtained from photographs taken with reduced exposure times at all three wavelengths showing that the dark artefacts no longer occur.

At low light levels, the processed image can either have too much noise for ready detection of bloodstains or can have dark areas which are not associated with bloodstains (as is seen in deep folds in clothing photographed using this technique). The darkness in the processed image is either being caused by breakdown in the linearity of the images or possibly due to stray non-monochromatic light dominating in areas shadowed from the monochromatic light source. Again, if necessary, a mask of very dark areas can prevent false identification of potential bloodstains. Areas that are too dark are particularly likely to occur on three-dimensional objects such as shoes. Increased noise at low light levels can be remedied by choosing an optimal ISO setting, increasing the exposure time (if necessary using additional noise reduction settings on the camera), increasing the illuminant intensity, or digitally filtering the image as described earlier. Taking images at different camera and/or lighting angles can be used to help identify these exposure artefacts.

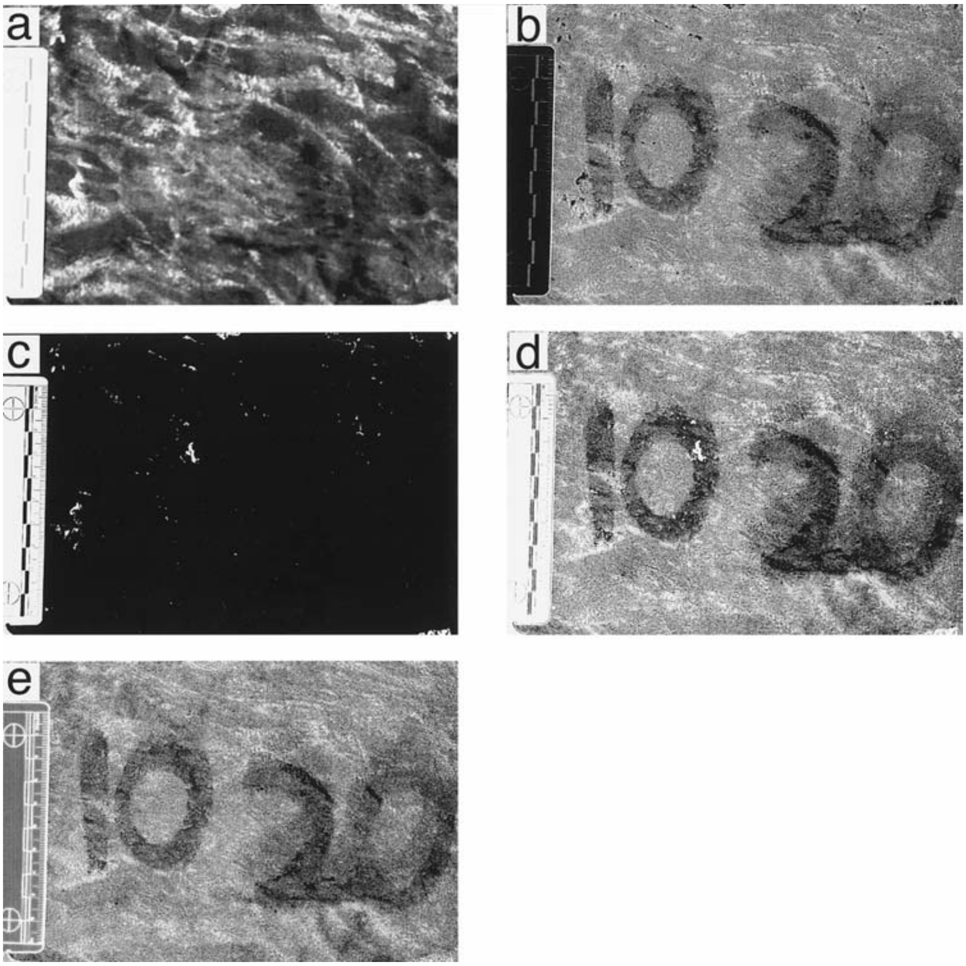

FIG. 5—*Artefacts caused by overexposed image.* a) *Overexposed image taken at 415 nm showing bright highlights.* b) *Processed image (using Eq 1) showing false blood (dark specks).* c) *Mask based on pixels with brightness levels greater than 140.* d) *Image obtained by applying mask to image* b). e) *Processed image obtained from photographs obtained at correct exposures.*

Detection Limits for Blood

The detection limits for the technique will differ for different camera settings, substrates (including the shape, texture, and coloration), and lighting. Under optimum conditions of uniform lighting and an even, light colored, substrate the detection limit will be limited either by the resolution of the imaging system or by the noise inherent the camera. If a limit of three times the standard deviation of the background noise is set for detectability (i.e., signal to noise ratio $= 3$) this would mean a difference of 3 levels at a level of 100 (for an ISO setting of 200 and an exposure time of $<1/10$ s), corresponding to an absorption of 3% of the light. Such a detection limit definition is appropriate if it is desired to identify a single pixel as representing a bloodstain. In practice, visual averaging of large features leads to a decrease in the perceived variability and an improvement in the detection limit (under some conditions, to displayed signal:noise ratios under 1 for features covering many pixels or with regular geometric forms) (6,8). Actual detection limits will depend on additional factors such as the viewer, viewing mode and quality (e.g., on monitor or print), and size of bloodstain. With these caveats in mind, experiments on bleached cotton showed that the lightest bloodstain detected using the three wavelength correction method corresponded to 1 in 900 diluted blood. As the substrate becomes more textured or more patterned, the ability to detect blood decreases so that on some substrates blood diluted twenty times is difficult to detect. If the color of the substrate was uniform then a two-wavelength correction (i.e., ratioing linear images taken at 415 nm and 435 nm) gave detection limits about a factor of two lower than that for the three wavelength method. This apparent worse detection limit for the three wavelength background correction method is most likely due to two factors: a) the nominal 395 nm image obtained with the system used in this paper actually has optimum sensitivity at a slightly higher wavelength due to the low sensitivity of the blue pixels in the camera towards light of wavelengths <400 nm, and b) the 415 nm Polilight band is sufficiently wide (full width at half maximum height (FWHM) $= 35$ nm) that it is not completely absorbed by the heme Soret band (FWHM ca. 25 nm). The combination of these factors leads to blood giving more similar reductions in pixel brightness in the 395 nm and 415 nm photographs than would be predicted from the absorption spectrum of blood. However, on patterned substrates the background correction possible with three wavelengths greatly exceeds that possible with two wavelengths so that the three wavelengths method has the better detection limit for blood under most conditions.

Other Artefacts

We have sometimes found that thick areas of undiluted blood either do not appear very dark in the processed image or even appear lighter than other areas, as shown in Fig. 6. This is most likely due to specular reflection from the surface of the blood, and this effect was also seen upon visual examination of the blood on this surface under the conditions used for the photography. Appropriate diffuse lighting may reduce this effect. In the particular example shown, the blood was instead detected using the inhibition of the fluorescence of the carpet substrate, because this was reduced whether light was reflected specularly from the blood or was absorbed by the blood. In general, blood thick enough to cause these problems should be visible by eye, and photography at longer wavelengths may be possible instead.

The Moire patterns seen in processed images of evenly textured materials (Fig. 7) is a result of the interaction of the regular texture of the cloth interacting with the regular pattern of a component in the imaging system—most likely the sensor element. These patterns are regular, and so are unlikely to be mistaken for bloodstains. If the Moire pattern did affect determination of a possible bloodstain, then re-photographing the item after a rotation of the item relative to the camera would lead to a change in the Moire patterns without affecting bloodstains. Furthermore, adjusting the camera to

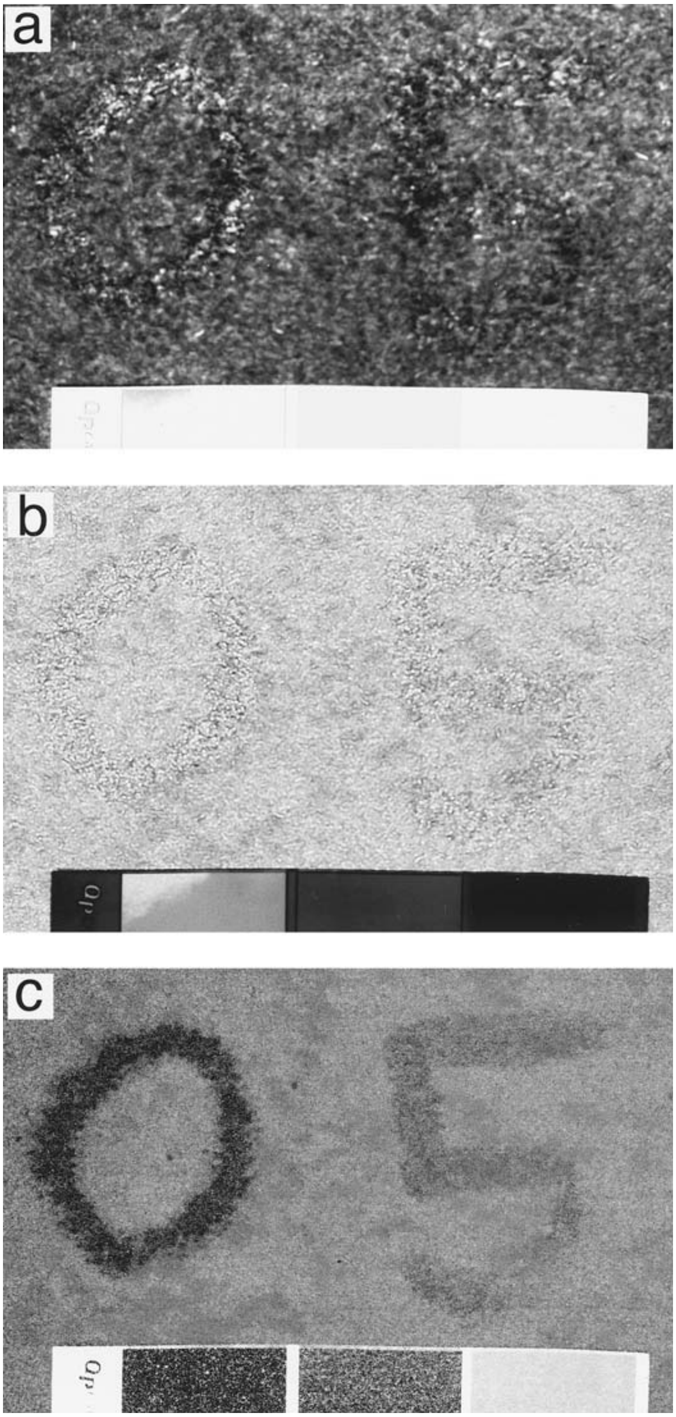

FIG. 6—a) *Photograph taken at 415 nm showing specular reflection from blood on a red carpet fragment.* b) *Processed image based on 395, 415, and 435 nm photographs, again showing effects of specular reflection.* c) *Processed image based on images obtained by photographing fluorescence emitted from carpet while exciting fluorescence at 395, 415, and 435 nm.*

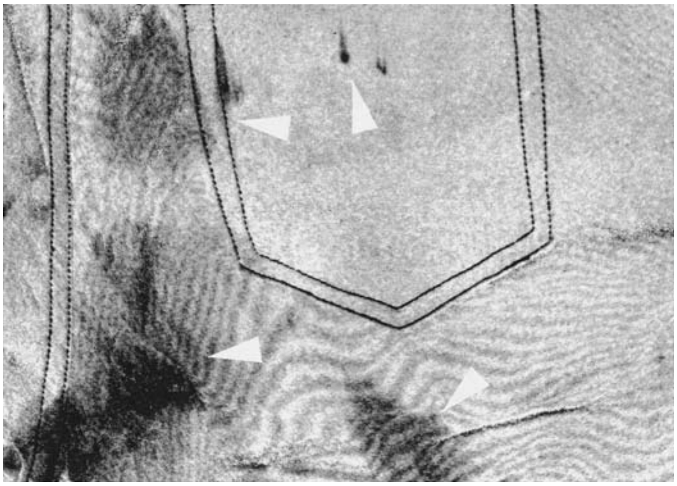

FIG. 7—*Enlargement of a region of a background-corrected image of bloodstained clothing showing bloodstains (arrowed) and Moire patterning (alternating dark and light regions).*

item distance can also change or reduce the Moire effect. Alternatively, if a program is available that can perform Fourier analysis of the image, then the periodic components causing the Moire effect can be removed from the image (7).

Use of Filters on the Camera

Two disadvantages of using an alternate light source are that it is usually limited in the area it can illuminate with sufficient intensity and that for the background correction technique to effectively work the photography has to be performed in a dark room. An alternative strategy is to place filters on the front of the camera. This can either be done in conjunction with a monochromatic light source to reduce the need for darkening the room (as suggested for single images by Stoilovic (9)) or the filters can be used in conjunction with white light sources that potentially allow illumination of much larger areas. The use of narrow bandpass filters was examined using 50 mm x 50 mm filters from Edmund Scientific. Narrow bandpass filters were used at 415 and 435 nm, but a shortpass filter was used for the 395 nm region. This was because a bandpass filter in this region was significantly more expensive and because characterization of the Polilight—camera system showed that the fall-off in overall sensitivity of the system at short wavelengths would lead to an effective bandpass detection.

The filters did indeed lead to the ability to take photographs in the presence of additional lighting and still be able to perform a background correction as described according to Eq 1. The filters also removed any effects due to the fluorescence of substrates. However, a significant problem identified with the use of the filters used in this study was misregistration, or a slight displacement of images taken with filters. This can be due either to the filters not being exactly perpendicular to the plane of the camera sensor or to the filters being slightly wedge shaped.

Characterization of the camera-filter system showed that there was a small systematic offset due to the filter holder being slightly non-parallel with the sensor plane. However, the major displacement was due to the construction of the individual filters. The result of this displacement of images relative to one another was the "three-dimensional" appearance of an image resulting from the combination of photographs taken with two or more different filters. This displacement effect also occurs when images using filters from widely separated spectral regions are combined (as can be done in examination of documents for alterations or fingerprints) (10). The effect of the displacement can be reduced by including an alignment target in the photographs and then aligning the images in an image processing program prior to mathematical processing. However, with at least one of our filters, the displacement was not constant over the whole image so that this method may not completely remove the "three-dimensional" effect. The consequence of this displacement on strongly patterned or textured substrates is that the edges of the pattern or texture retain strong contrast so that the visibility of bloodstains is reduced compared with what would be observed if the images were in perfect registration. Application of a Gaussian blur reduces the edge effects, but the background removal in such images is still not as good as in those taken with no filters on the camera. Even so, the bloodstains in processed images derived from photographs obtained with filters on the camera are still significantly better defined than if a single photograph taken under white light or with a single alternate light source band was examined. It is possible that higher quality filters in a filter wheel could overcome or reduce this misregistration problem.

Use of Non-Linear Processed Images

Most of this paper and the previous paper focus on the use of linear images—those in which the pixel levels are proportional to the intensity of the detected light. There are comparatively few consumer digital cameras that are able to export such images, despite the fact that the sensors in the cameras are linear detectors. Most cameras export images that are in non-linear form because such images are more similar to those obtained from traditional film photography. Such images are also often compressed, usually in jpeg format. We have not investigated the effect of compression on the background correction technique but we have examined the behavior of uncompressed non-linear images under this technique. As noted in the opening section of this paper, the close-to-logarithmic nature of non-linear images means that the background correction should be performed by subtracting images, rather than ratioing as is done for linear images. Although the subtractive background correction according to Eq 2 does provide increased detection of blood compared with a single photograph, it is not as good in general as the method using linear images and Eq 1. Possible reasons for this are the fact that the non-linear processing is not exactly logarithmic or that the nature of the logarithmic process means that bright pixels are not as well differentiated as in a linear image. Furthermore, the subtraction of non-linear images does not seem to remove backgrounds involving different colors as effectively as the linear ratioing. The advantage of non-linear images is that cameras producing such images are much more widely available than cameras that can provide linear images, and that lower-cost cameras are usually only able to export non-linear images. In addition, the processing of such images only requires averaging and subtraction of images, and these operations are available in more image processing programs than is division. These advantages must be weighed against the worse detection limits and decreased removal of patterned backgrounds possible with non-linear images.

Use of 8-Bit Images

As noted above, the noise in a consumer digital camera can be sufficiently large that at pixel brightness levels above 60 (in a linear image), the variability is greater than ±1 and therefore 8-bit images and processing might be expected to give similar results to 10-bit or higher images for well exposed photographs. The advantages of the higher-bit images are seen in the greater available dynamic range (in this case, giving correct response at low pixel levels in an image) or if pixel averaging is performed (either digitally or visually). Indeed, if the illumination of an subject was relatively uniform, the substrate did not differ greatly in reflectance over the field of view, and the photographs were well exposed, then the results obtained using 8-bit processing were very similar to those obtained using higher bit resolutions for the camera used in this study. The only precaution to be taken if 8-bit image processing is used under such conditions is that image division must be accompanied by a mathematical operation to spread the calculated pixel levels over a "reasonable" range (e.g., 0–100), otherwise posterization (i.e., a limited number of greyscale levels) will be observed. If the light intensity is non-uniform or the substrate has darker areas, then 8-bit processing may cause artefacts in the darker areas, in which case exposure bracketing may be required so that different areas of the subject are exposed correctly in different image sets. The advantage of 8-bit image processing is the more ready availability of cameras and programs with this bit resolution. This must be weighed against the greater flexibility offered by 10-bit or greater systems.

Discussion

The background correction of photographs can be a useful technique in the forensic context when used appropriately. If a substance (such as blood) has an absorption band in the visible region which is narrower than the absorption bands of common substrates and colorants then this technique can aid in the detection of the substance. The detection of blood is most readily accomplished if the substrate is of uniform color, the lighting is uniform, and the camera is relatively noise-free. Under conditions where these conditions are not met, detection of blood will be more difficult. This paper suggests limits within which blood detection using this method can be used reliably. Of course, it must be stated that the background correction method does not definitively identify blood—it is still necessary to perform confirmatory tests on representative stains. On the other hand, this method can identify regions where blood is likely to be found and delineate the area of the bloodstaining.

The camera characterization section identifies methods for ascertaining whether a particular digital camera might be suitable for using to generate images for background detection. Most consumer digital cameras are optimized for visual examination of single images, and their specifications do not necessarily match those of scientific digital cameras. The limits due to the camera are both due to noise in an individual image and variability between images. Inter-image variability can be corrected by including a neutral density target in each image and then multiplying (or dividing) images by a constant to correct for the variability based on the brightness of the target. Within-image variability is due to a combination of read noise, dark noise, shot noise, and, potentially, fixed pattern noise. The major cause of inter-pixel (within-image) variability for the camera used in this study was shot noise. This noise is a function of the depth of pixel wells (number of electrons that can be stored in each pixel of the detector sensor) and the gain of the sensor pixels, since the relative standard deviation of shot noise is equal to the ratio of the square root of the number of detected photons to the number of detected photons (4). The gain of the sensor on a consumer digital camera is controlled by the ISO setting, so increasing the ISO setting from 100 to 400 increases the gain by four and thus increases the shot noise at a given image pixel intensity by a factor of two. The combined read noise and dark noise is comparatively low for the camera investigated in this study at low ISO settings and short exposure times. However, for ISO settings of 400 and above and exposures of over 10 seconds the read and dark noise was significant (Table 2). The Canon D30 has a function designed to reduce this noise, while more recent cameras such as the Canon D60 have reduced this noise contribution.

The shot noise for a given digital camera can be estimated by taking a picture (in linear mode) of a uniform image under conditions where the mean pixel brightness is between 50 and 100. The inter-pixel variability in such an image is likely to be dominated by shot noise. The standard deviation of a selection of pixels (e.g., a 50×50 region) is then an indication of the shot noise. The mean brightness level of the selected region divided by the observed variance (i.e., (standard deviation)^2) will provide an estimate of the number of detected photons per brightness level. The relative shot noise at a particular pixel brightness level will be lower a) for a camera with a larger number of detected photons per brightness level (and therefore "deeper well depth"), and b) if a low ISO setting is used. This then is an additional figure of merit for a digital camera beyond the number of pixels in the camera. This analysis assumes that the dark/read noise is negligible compared with the shot noise, which was found to be a good approximation for the system used here under reasonable illumination and with low ($\leq$400) ISO settings. Again, the dark noise for a given camera under specific settings is readily determined by taking photographs with the lens cap in place.

Processing of photographs taken at three wavelengths according to Eq 1 or Eq 2 can lead to enhanced detection of blood as long as the pixels in the original photographs are at appropriate brightness levels. The upper and lower limits of pixel brightness have to be determined for each particular camera. Photographing a grey scale card gives a quick indication of the limits on linearity of a camera, although more extensive evaluations of camera linearity can be performed by taking photographs while varying the exposure time. The practical limits of the described background correction technique at low pixel brightness can be determined by examining processed images and identifying the pixel brightness levels in the original photographs that cause dark areas to appear even though no blood is present. If a set of images of a potentially bloodstained item includes pixels that are under- or over-exposed, there are two possible options: a) retake the photographs at different exposure settings or lighting conditions or, if that is not possible, b) use the thresholding tool in an image processing program to create a mask eliminating the overexposed (or underexposed) pixels from the processed image.

The use of narrow bandpass filters on the camera increase the usefulness of this technique by allowing photographs to be taken in the presence of ambient light and also under white light illumination. This in turn can allow the technique to be used to image a large area such as a wall. The use of filters can lead to problems in the image processing due to slight mis-registration of images taken with different filters, but this effect can be reduced by using an image processing program to bring the separate images into better registration. An additional problem with the use of traditional narrow bandpass filters is that a large selection is needed if more than one region of the spectrum is going to be analyzed using this background correction technique. Recent developments in filter technology (especially the development of electro-optical filters) mean that hopefully soon affordable tunable filters can be used in this application.

In conclusion, this paper provides practical guidelines to the selection of a camera appropriate for use with the three-wavelength background correction technique and identifies the limitations and potential problems associated with using this technique. The concept is not limited to photography of blood, and could be used for any other substance with a narrow visible absorption band if an appropriate light source or filter combination is available.

Experimental

The camera used was a Canon D30 digital single lens reflex camera, with either a Canon 24–85 mm zoom lens or a Canon EF 100 mm F2.8 mm macro USM lens attached. This camera has a CMOS detector with 2160 × 1440 active pixels for a total of 3.11 M pixels, and can output files with a bit depth of 12. The camera was mounted on a camera stand or tripod and was activated from a computer using the programs Remote Capture (Canon) or D30Remote (Breeze Systems) to avoid camera motion and thereby ensure registry of the images. Alternatively, registration points could be provided on each image to assist alignment. In a forensic context this is readily achieved by including scales in all pictures. Photographs were made in a black box or in a dark room unless bandpass filters were being used on the camera. For most examples discussed in this paper focusing was done manually, the aperture was set at the maximum for each lens, and the camera autoexposure function selected the most appropriate shutter speed. A cap was placed over the viewfinder after the lens had been focused to prevent inaccuracies in the exposure calculation. This was particularly important for the 395 nm photographs. Setting the aperture to the maximum lead to a reduction in the depth of field of images, but kept the light level to the sensor high (this was an important consideration for the 395 nm images). The detected light intensity using the Polilight—Canon D30 combination decreased by approximately 100 times from 435 to 395 nm, so variable shutter speeds were used to obtain similar integrated light levels for each photograph (see previous paper for justification of this approach). Substrates were chosen to represent a range of possible fabrics, colors, and patterns.

The light source used was a Rofin Polilight alternate light source, which consists of a xenon lamp, narrow bandpass filters, and a liquid light guide with a glass collimator lens. The filters have nominal bandwidths varying from 40–80 nm, although our particular instrument now has slightly degraded performance for two of the filters. The most appropriate settings for obtaining light centered around 395, 415, and 435 nm were determined by monitoring the Polilight output with a laboratory spectrophotometer. For some experiments narrow band filters were placed in front of the camera lens. A Cokin filter holder was fitted with custom-built adapters to hold 50 mm × 50 mm square interference filters from Edmund Scientific. Narrow-pass filters centered on 436 and 415 nm were used in this study. For the short wavelength image, a U360 shortpass filter was used since the reduced light output from standard illuminants and the reduced sensitivity of the camera at short wavelengths meant that it was not necessary to use a bandpass filter. Characterization of filter transmission, Rofin Polilight output, and substrate reflectivity was performed using an S2000 Ocean Optics fiber optic spectrophotometer.

Most images were collected in the RAW mode on the Canon D30 camera. Files were either transferred directly to the computer or saved on a 64 MB card in the camera and then transferred to the computer using the program Canon ZoomBrowser. The raw files so obtained are in a proprietary 12-bit format. These were then converted into 16-bit TIFF files using Canon ZoomBrowser, with the conversion set to linear mode and with the white balance set to flash or cloudy (these were the closest white balance settings to the white light of the Polilight).

The TIFF files so obtained were then processed in Adobe Photoshop 6.0 (Adobe Systems) with the Fovea Pro 2.0 (Reindeer Graphics) suite of plug-in filters. The Fovea Pro filters were used to perform calculations with the 16-bit images because many filters in Adobe Photoshop only act on 8-bit images and the Fovea Pro filters provide greater mathematical flexibility. In accord with Eq 1, the 395 and 435 nm images were averaged, and the 415 nm image was then divided by this averaged image. Division of two identical images will lead to a value of 1, so each pixel value was then treated mathematically to spread the obtained image in intensity space. The averaging and division by Fovea Pro are performed independently on the three channels (red, green, and blue), so it was not necessary to isolate the required channel at this stage. The blue channel was then isolated and the contrast of the blue channel of the obtained image was adjusted using the Levels command in Adobe Photoshop® to provide optimum contrast of the bloodstain compared with the substrate. In most cases this led to an almost complete removal of background patterns and a consequent enhancement in the detectability of blood. For some comparisons, non-linear images (the normal images obtained from consumer digital cameras) at the three wavelengths were also combined. In this case, the image obtained by averaging the 395 and 415 nm photographs was subtracted from the 415 nm image in accord with Eq 2. Finally (although no images are shown in this paper), some images were examined after background correction of the 415 nm image using only the 435 nm image. On uniformly colored substrates this led to improved results compared with single wavelength measurements and gave slightly lower detection limits for blood, but on patterned substrates this two-wavelength method was not as good at background correction as the three wavelength method. Bovine blood containing 5 g/L EDTA was stored at 4°C. The concentration of hemoglobin was determined to be 124 g/L (11).

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