Visual Appearance and Chemical Detection of Bloodstains on Concrete After Exposure to the Elements

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Abstract

We report on the experimental detection of blood on exposed concrete over a period of 428 days in the city of Aurora, Colorado. Neat (undiluted) blood was poured in an x- shaped pattern in each grid unit. The x-shaped pattern was chosen as a means of validating any reaction with possible false-positive properties of the substrate as well as to gauge how much deterioration of the pattern may occur over time, if any. Visible luminol reactions of the x-shaped pattern were detected from 14 to 156 days of exposure to the elements. A novel technique for extending this time frame using image enhancement is also discussed which extended the detectable reaction to 295 days.

Keywords: Blood detection, luminol, image enhancement, crime scene reconstruction, forensic science

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Introduction

Luminol is a presumptive blood reagent used to detect trace bloodstains. The authors selected this reagent because its sensitivity is known to be one part per million making it one of the most sensitive of the blood reagents available [1, 2]. When luminol is exposed to blood, it reacts with the iron-containing heme group of hemoglobin and its derivatives to produce a faint blue/green chemiluminescent reaction [2]. Chemiluminescence is the emission of light from a chemical reaction. This reaction can be photographed using a film or digital camera, tripod, and a long exposure time in a darkened location. Luminol has been used in forensic applications at least since 1937 [3] and comes in various modern trade names and proprietary formulations to include BlueStar, Hemascein, Lumiscene, and Lumiscene Ultra. The latter two combine luminol with another chemiluminescent reagent, fluorescein. Though fluorescein produces a chemiluminescent reaction, it differs from luminol because it requires the use of an alternate light source and an appropriate filter in order to visualize [2].

In prior studies, researchers tested the sensitivity of luminol reaction to bloodstains that were left for extended periods on soil [4, 5, 6, 7]. One previous case report of bloodstain detection on concrete tested out to 72 days

following deposition [8]. As noted in a prior study, the reactivity of luminol improves as a bloodstain ages. This reactivity is due to the breakdown of the blood and exposure of the heme group [2]. It is also known that under ideal conditions, blood over a century in age has reacted positively to luminol. In this study, the authors set out to determine the physical appearance of bloodstains on concrete over time and the duration bloodstains could be detected on concrete using luminol. The bloodstains were fully exposed to the elements in a public area of the Denver Metropolitan area for almost 15 months. The stains were periodically examined and tested using luminol to observe changes in sensitivity and identify the time frame necessary to render the stains undetectable. These results were then compared to the results of other similar studies.

Materials and Methods

The test site was a concrete slab located on the west side of a two-story building in Aurora, Colorado on the campus of the University of Colorado Hospital. The test site was shaded during the early morning hours and subjected to full sunlight the remainder of the day because there were no other nearby structures to provide shade.

Meteorological data consisting of daily minimum and maximum temperatures, humidity, and precipitation were obtained from the Aurora Hills weather station located approximately 4 miles (6.5 kilometers) from the test site. Temperature and precipitation are plotted in Figures 1 and 2, respectively.

The test site was prepared by constructing a grid with individual grid squares measuring one foot square (30 × 30 cm). The grid squares were marked with string suspended between nails placed along the periphery of the test site. The overall test site area was 99 ft^2 (9.2 m^2).

To prepare the test grids, on July 26, 2010 (day 1) each test grid was stained using 10 ml of whole horse blood. The stain was made in an x-shaped pattern to control for false-positive reactions and gauge any pattern degradation during subsequent testing. The surface of the concrete slab did contain some irregularities such as small chips and craters. When applied, the blood naturally conformed to these surface irregularities giving each individual grid square and bloodstain pattern a unique appearance. The primary test grids consisted of 88 grid squares. This number of grid squares were prepared so that each time tests were conducted a fresh grid square could be used.

Initially, there was also a control grid composed of 80 grid squares that were treated in the same manner at the primary test grids. The intent was to shield the control grid from full exposure to precipitation by covering it with a tarp to prevent direct exposure to rain and snow. However, the strong winds associated with Colorado summer storms soon worked against this plan by repeatedly blowing the tarp off the precipitation control grid despite the use of heavy stones to weigh it down. As a result, the authors decided that any comparative value the precipitation control grid might have had was compromised and its further use in the study was abandoned.

Over the following 15 months, the authors periodically documented the appearance of the test grids under normal lighting and processed grids with luminol. The luminol was prepared within two hours of use. The formulation used for all luminol reagent treatments was:

- 0.5 g 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol)
- 25 g sodium carbonate
- 3.5 g sodium perborate
- 500 ml distilled water

Prior to each application, a known source of blood was tested as a positive control to ensure the reagents were working properly. An area of the concrete slab outside the testing grid was also treated with the reagent to test for any false positive reactions. No false positive reactions were developed.

Photographs were taken using a Nikon D2x digital single lens reflex camera with a Nikon 17-35 mm *f*/5.6 wide-angle zoom lens. The luminol exposures were timed using a watch and an electronic shutter release with the camera on the bulb setting and supported on a sturdy aluminum tripod. To minimize the effect of artificial light contamination from nearby light posts, the camera and tripod was covered with an opaque cloth, however, minor light leaks were unavoidable. All luminol exposures were done with the aperture wide open at *f*/5.6. The length of the exposure was varied depending on the strength of the luminol reaction, which

will be discussed in further detail below. These tests were repeated until there was no detectable reaction to luminol.

A Sony model DCR-HC28 video camera with "Night Shot Plus" low light technology was also tested to capture the luminol reaction, but abandoned after very poor results.

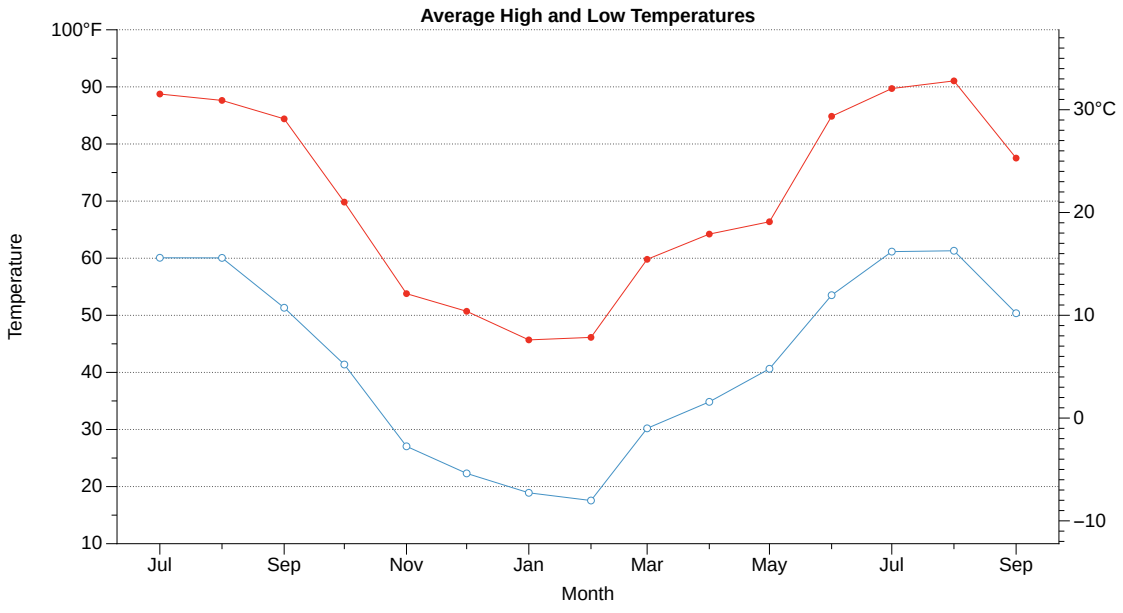
Weather Conditions

The authors were unable to locate previous published studies on the direct results of precipitation and temperature to the rate of dilution and degradation of bloodstains on concrete in outdoor environments. It seems apparent that precipitation can dilute bloodstains and changing temperatures can break down molecular structures. In this study, the blood was poured onto a porous surface and left unprotected from normal weather conditions over all four seasons. The concrete proved to be relatively non-absorbent surface. The original deposition of blood stayed predominantly on the surface until subjected to further dilution by rain. It is unknown if the duration of detectability would change if the testing area were located in areas subject to consistently higher or lower temperatures, humidity, and precipitation. During the course of this study, the testing area received less than 19 inches of precipitation. Figures 1 shows a plot of the average high and low temperatures during the test period. Figure 2 shows the monthly precipitation.

Results

Visible Stains. As the original bloodstains faded and were washed off by rain, they left behind an outlined image of the original x-shaped bloodstain. This appears to indicate that despite the volume of blood present, the stain failed to penetrate the concrete immediately below the original deposition. However, as the rain washed the blood away from the original stain, the diluted blood stained the surrounding concrete. This resulted in an outlined image of the original stain (Figure 3).

With time, the weathering washed away the original surface deposition of blood and the stains left in the concrete became increasingly faint. Figure 4 shows the progression of grid 5M from day 1 to day 376 under normal lighting. The visits to the site were more frequent during early stages of the study when the visible changes to the pattern were more rapid and became less frequent as the difference was less dramatic. By day 6, the dried blood volume on the surface of the cement had already largely washed away and left only the outline described above. This outline persisted until day 95, by which time there was only a faintly visible outline remaining. Under normal lighting a ghost image of the stains did persist until the end of the study at day 428. However, for all practical purposes those stains were only recognizable because of their repeating pattern and because their location and size were known



◀ Figure 1: Average monthly minimum and maximum temperatures during the test period.

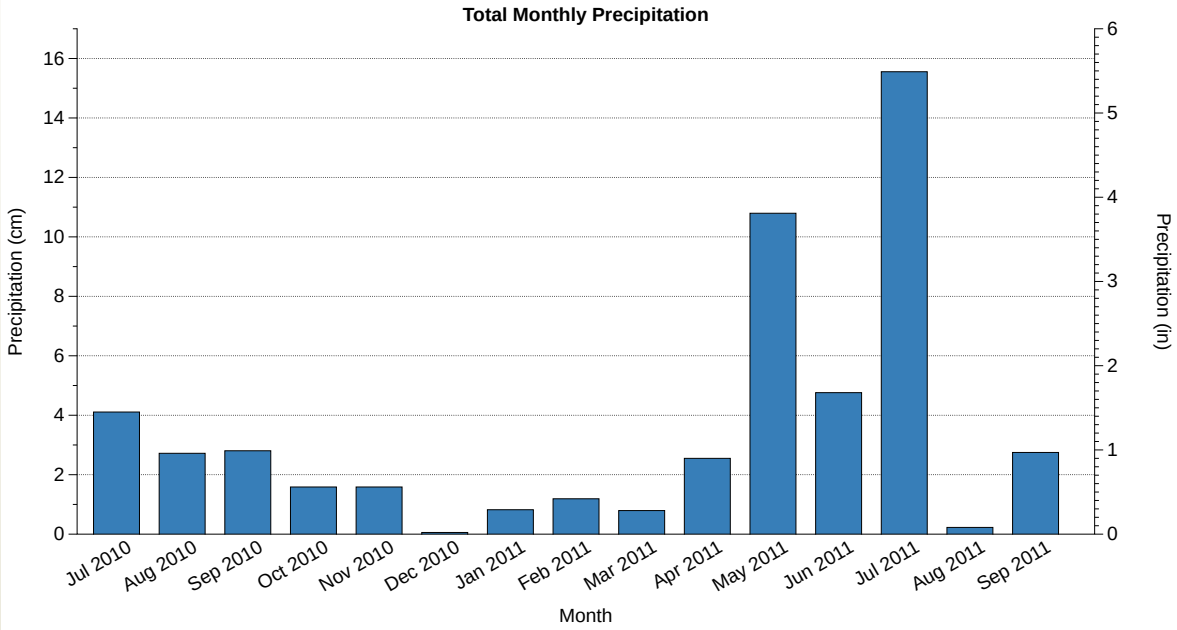


Figure 2: Total monthly precipitation during test period.

to the authors. Recognition of these stains as blood, under normal lighting in a true crime location would become difficult soon after the surface stain was washed away, by day 6 in this study. Realistically, in a crime scene the variable shape and distribution of various bloodstain patterns and other non-blood staining agents would only further complicate their visual detection.

The rapid visible changes to the stains early in the study are largely attributable to the rainfall they were being exposed to during the first 15 days of the study. Table 1 shows the daily rainfall for days 1 through 15.

Luminol Reactivity. Visible luminol reactions were observed beginning on day 14 until day 295. However, the original x-shaped stain was only apparent until the testing done on day 156. These reactions became weaker with time and required increasingly longer exposures to document photographically. At day 295, there were only faint spot reactions.

At day 375, a visible reaction could no longer be detected photographically, although attempts continued through day 428 with exposure times up to 310 seconds with no apparent results. It was later determined that image enhancement (discussed below) could extend the detection of the x-shaped reaction to day 295.

Extension of Photographic Detection through Image Enhancement. It was discovered that the very faint and seemingly blank images from day 295 to 375 did contain

Figure 3: The image of grid 6L on the day the blood was deposited (a) compared with the same grid on day 5 (b) after having been rained on. This illustrates how the original stain was outlined by the diffusion of the stain into the surrounding concrete. A careful comparison will show that the outline is true to the original shape of the stain and retains many fine details.

◀ Figure 4: Appearance of bloodstains in grid 5M from day 1 to day 376. Starting on the upper left and proceeding by rows, the images are for: Day 1, Day 2, Day 4, Day 6, Day 9, Day 10, Day 13, Day 15, Day 26, Day 34, Day 43, Day 95, Day 158, Day 376.

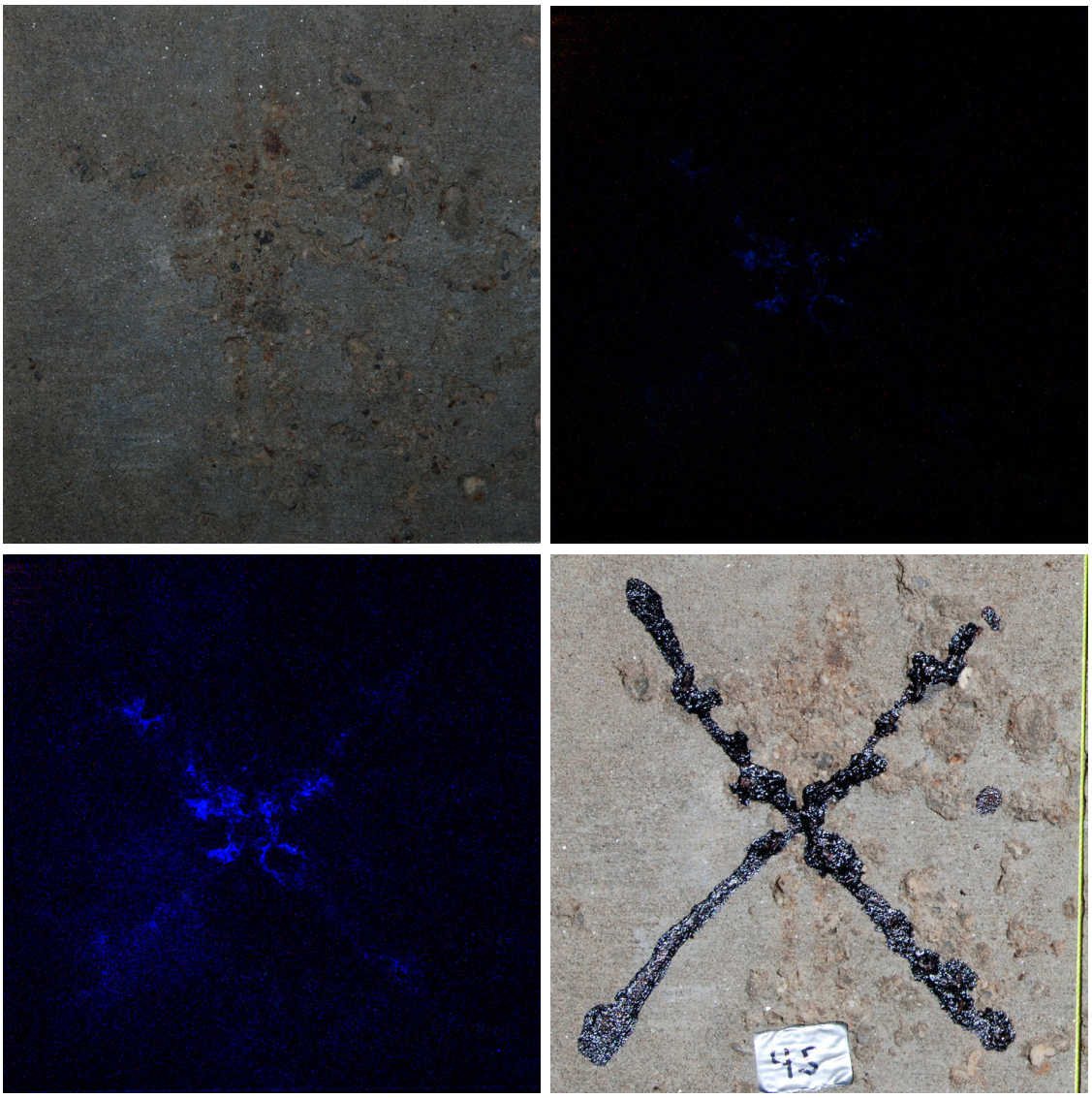
Table 1: Daily rainfall totals from day 1 to 15.

Day	Rainfall (cm)	Rainfall (in)
1	0.00	0.00
2	0.25	0.10
3	0.00	0.00
4	0.00	0.00
5	0.33	0.13
6	0.64	0.25
7	0.00	0.00
8	0.00	0.00
9	0.08	0.03
10	2.11	0.83
11	0.08	0.03
12	0.00	0.00
13	0.00	0.00
14	0.00	0.00
15	0.03	0.01

faint exposures from the luminol reaction that were not discernible in the image without additional post-processing. It was also found that the visible reactions from day 156 could be significantly enhanced using the same technique. The use of image processing software to enhance evidentiary photographs in crime scene investigation can be subject to challenge since it could introduce artifacts. However, in this case the authors believe there is evidence to suggest that image processing of chemiluminescent reactions can be scientifically sound and a specific procedure is suggested.

Figure 5a shows an image of grid 7V taken on day 46 under visible light. The exposure in Figure 5b was 5 minutes long, yet there is no apparent reaction visible that could be associated with the known stain. When post-processing in Adobe Photoshop using the levels tool (Figure 5c), the chemiluminescent reaction becomes very clear and distinct. Because the original stain was documented

Figure 5: The upper left image (a) shows grid 7V on day 46 under visible light. The upper right image (b) shows the same grid while processed using luminol under a long exposure. The lower left image (c) is the same as the second image, but has been processed in Adobe PhotoShop using the levels tool to reveal the faint luminol reaction. The lower right image (d) shows the original deposition of blood as photographed on day 1 for comparison to the luminol results.



◀ Figure 6: The upper left image (a) shows grid 4S on day 295 under visible light. The upper right image (b) shows the same grid while processed using luminol under a long exposure. The lower left image (c) is the same as the second image, but has been processed in Adobe Photoshop CS6 using the levels tool to reveal the faint luminol reaction. The lower right image (d) shows the original deposition of blood on day 1 for comparison to the luminol results.

when produced, we can compare the enhanced image to the original visible bloodstain from day 1 (Figure 5d). This side-by-side comparison clearly shows that the enhanced image is revealing actual data recorded by the camera sensor from the luminol reaction. The shape and size of the original stain are clearly visible and refute the possibility that the image enhancement is producing some type of artifact or enhancing data unrelated to the luminol reaction. However, the diffusion of the blood around the original stain, as discussed earlier, creates a background that would not be easily distinguished from that of the original stain without the prior photographs for comparison.

This image enhancement technique was used to reveal the x-shaped pattern on an image taken up to 295 days after the original deposition. This can be seen in Figure 6, which shows grid 4S on days 1 and 295 under normal lighting, the unenhanced photograph of the luminol reaction exposed for 340 seconds, and the same image enhanced to reveal the x-shaped reaction. After 295 days, there were areas that showed a blue glow, but these reactions no longer showed the x-pattern of the original bloodstains.

The enhanced image at 295 days is much less complete than the previous example. However, the overall x-shape is visible and close inspection will reveal some details of the original stain's shape are still discernible.

The authors would caution that these results was not the intent of the study and may not be sufficient to support the use of this technique in actual casework. Nonetheless, the results are highly encouraging and support the idea that enhancement of images of chemiluminescent reactions could be a valuable tool if fully investigated and documented to fully identify

its limitations. The technique could also be used on images with visible chemiluminescent reactions to bring out additional details.

it. The importance of the adjustment being on its own layer is that any changes made on that layer are not directly altering the original image

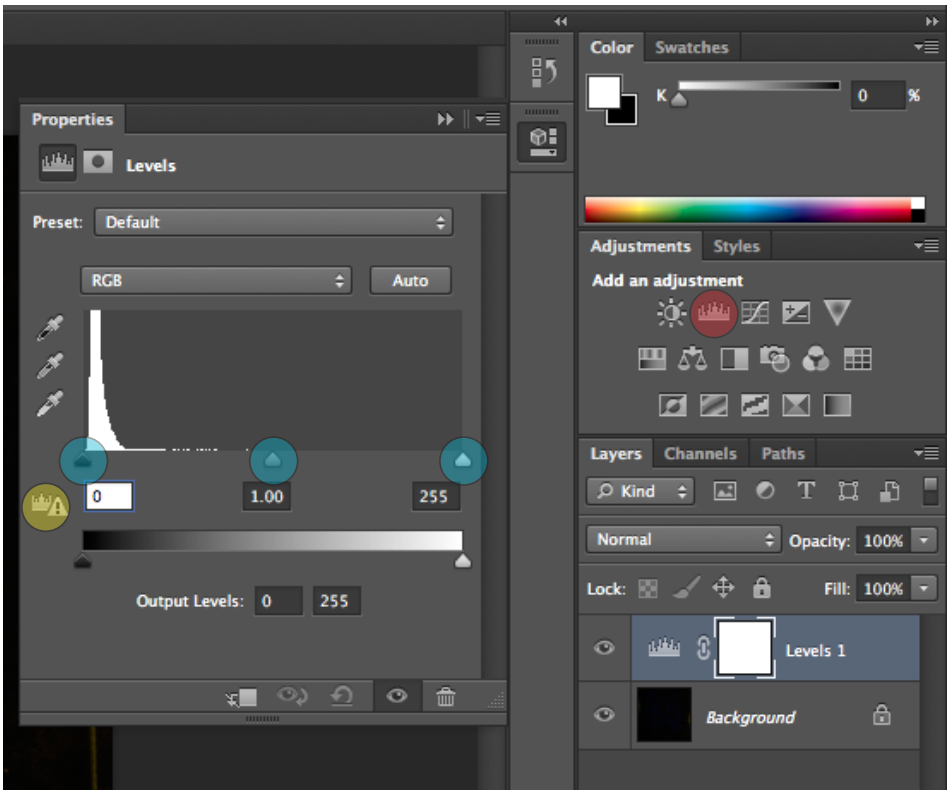


Figure 7: A cropped screen shot from Adobe Photoshop showing the adjustments, layers, and levels tool. The levels selection icon is highlighted in red. The icon highlighted in yellow calculates a more accurate histogram. The slider controls on the histogram are highlighted in blue.

Recommended enhancement procedure. After experimenting with various options, the authors found that the simplest method for enhancement in Adobe Photoshop CS6 was to make an adjustment layer using the levels tool. Figure 7 shows a cropped screen shot from Photoshop with the relevant tools highlighted for reference. To make the enhancement, open the desired file in Photoshop. From the Adjustments menu, select the Levels tool (highlighted in red). This creates an adjustment layer that can be seen in the Layers menu below in the background layer. This means that the adjustment layer can be turned on, turned off, or deleted without altering the original image.

The levels tool that appears to the left of the adjustments tab is what is then used to make the enhancement. First, click on the icon highlighted in yellow that calculates a more accurate histogram. The histogram is the graph that dominates the levels tool window. This graph plots the intensities of colors along the y-axis and the tonal range along the x-axis. The tonal range runs from zero (pure black)

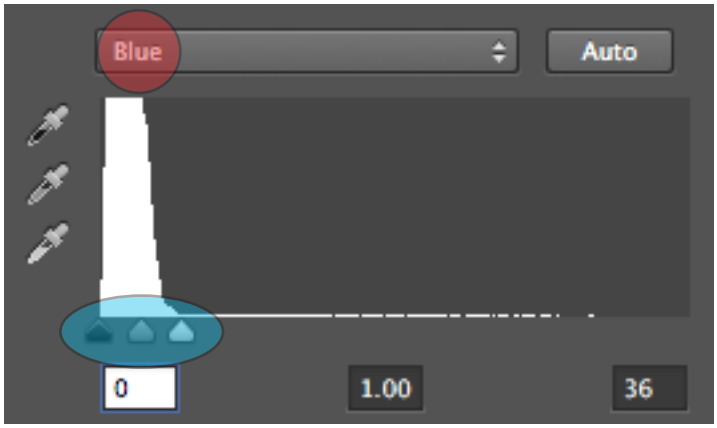
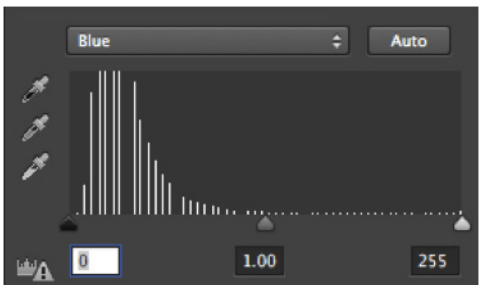


Figure 8: A cropped screen shot showing how the levels tool was used for image enhancement. The histogram was changed from RGB to Blue, which was highlighted in red. The right most slider was moved to the right side of the histogram, which was highlighted in blue.



◀ Figure 9: A cropped screen shot showing the resulting histogram after being linearly expanded across the entire tonal range.

to 255 (pure white). Because the image being enhanced was predominantly dark, the graph in Figure 7 is weighted to that side of the graph. The remainder of the histogram indicates little or no pixels at those values.

By default, the histogram is displayed in RGB (red-green-blue) mode. It was found that selecting the blue layer from the pull-down menu (highlighted in red) produced better results since the light from the luminol reaction is primarily bluish. Finally, when adjusting the histogram sliders highlighted in blue (Figure 7 and Figure 8), the goal is to eliminate the portions of the histogram that lack color data. In the case of the example image, this was accomplished by simply sliding the right most slider until it met the right edge of the histogram (Figure 8). The adjustment then mathematically maps all the values between the sliders across the entire tonal range from 0 to 255 [1]. The values between the sliders are linearly expanded across the entire tonal range. This is what results in higher contrast image and makes the faint luminol reaction visible against the background.

The result can be seen in the post-adjustment histogram (Figure 9), which shows what is sometimes referred to as a combed histogram due to its resemblance to the teeth of a hair comb. The gaps in the histogram are the result of the expansion of a narrow range of data. All values at or below the left slider are mapped to pure black (0) and all the values at or above the right slider are mapped to pure white (255). This does mean that a small number of pixels outside the sliders could be mapped directly to 0 or 255, resulting in the loss of that data. However, if adjusted properly the lost data should represent only a very small number of pixels and should not significantly affect the final image. In fact, truncating a few very high value outlying pixels is typically necessary in order to obtain the best contrast for the bulk of the pixels. This is why the sliders need to be manually adjusted to select for the bulk of the values in the histogram as opposed to clicking on the auto adjustment. The auto adjustment does not truncate any pixels, resulting in a lower contrast adjustment that will likely be insufficient for these purposes.

This technique was also successfully accomplished using GIMP 2.8 (GNU Image Manipulation Program), a freely distributed program. The only noteworthy difference is that GIMP does not perform the levels adjustment in a separate layer, therefore altering the original image. This should not be a problem as long as the enhanced image is saved under a new file name and the original image is maintained. GIMP is available for download from www.gimp.org with support for Microsoft Windows XP and Vista, Linux, and Mac OS X operating systems.

Though the technique described above proved effective for the authors, other researchers may discover other useful techniques. Additionally, other image editors may offer different tools. Full-resolution, unenhanced tif files of Figure 5b and Figure 6b are being included with the Supplemental Materials for this article. The images will be available on the ACSR website (www.acsr.org) for anyone who wishes to download them to try the above described image enhancement themselves or attempted other enhancement techniques.

Discussion

Comparison to Prior Studies. A commercial fertilizer containing dried blood was studied in Washington State [2]. The study found that the blood was detectable with luminol a year after being deposited. In an ongoing study being conducted at the Highlands Ranch Law Enforcement Training Facility south of the Denver metropolitan area, blood was deposited on soil and processed with luminol after 1, 2, 4, 6, and 8 years with visible positive results [4, 5, 6, 7]. It is noteworthy that after 24 months, the reaction on the surface soil had diminished and a better result was obtained by scraping away a thin layer of the soil before the application of luminol. It was proposed that this was due to filtration of the blood into the soil and/or wind erosion of the topsoil.

In our study, the detection of bloodstains

was not possible when attempted at 428 days, even with the application of image enhancement tools. The blood in soil studies described above and the current study on concrete were conducted within ten miles of each other and the weather conditions were very similar. The authors believe there are two likely explanations for why the bloodstains on concrete were detectable for such a short period compared to the prior studies on other mediums.

First, unlike the soil experiment, the authors could not just scrape off a layer of the concrete when a reaction was poor. This introduces the possibility that the stains could be penetrating deeper into the concrete, but any reaction to the luminol was masked by the concrete above. However, the porosity of concrete relative to blood absorption has not been reported in the forensic literature. It is possible that the bloodstains could not penetrate beyond the surface layer of the concrete and were thus more susceptible to the forces of nature than blood in soil.

Second, concrete has a higher capacity to absorb and retain heat than soil. As a result, it is possible that the increased heat and longer exposures to heat could be denaturing the heme group in the blood and rendering it undetectable to luminol.

Conclusions

Bloodshed is a common event during violent interactions. When bloodshed occurs outdoors, the blood is susceptible to a variety of environmental processes that can damage or destroy its evidentiary value. Delayed reporting or discovery of a crime scene adds to the destructive potential of these processes. This study documented the fading process of a 10 ml bloodstain on concrete and found that under the test conditions stains became faint outlines by about 3 months. These faint stains were visible under normal lighting but, no longer resembled bloodstains and could easily be overlooked by investigators had this been a crime scene. Therefore, it is important for investigators to note even faint discolorations in an old crime scene and conduct additional chemical tests for the presence of blood.

This study also demonstrated the feasibility of using luminol to detect blood on concrete that was fully exposed to the elements for at least 5 months after deposition without any need for image enhancement. However, after 5 months the reaction was too faint to be visible without image enhancement. This made proper photographic technique and controlling ambient light very important.

Chemiluminescent blood reagents, like luminol, are designed for application to small specific areas. Large outdoor search areas pose significant challenges to the crime scene investigator due to the need for application over numerous small test areas, light control, and cost. As the visible stains became faint and the luminol reaction less obvious, the need for reliable witness information about the specific location of a stain is increasingly critical.

Finally, the results of image enhancement by histogram expansion appear to be a reliable technique, if properly applied. Readers are cautioned that they would need to be prepared to explain and defend the nature of such enhancement if it is intended for use on actual evidence. We also encourage others to conduct similar experiments in their area to determine what climate and environmental factors may affect blood detection from outdoor environments.

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Supplemental Materials

The following data are available to download from the ACSR website.

- Spreadsheet with unabridged weather data for test period.
- Full-resolution, unenhanced images of Figure 5b and Figure 6b.

Disclaimer

The opinions expressed in this article are those of the authors and do not necessarily represent those of their employing agencies.

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