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TECHNICAL NOTE

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The Use of a Tunable Light Source (Mini-Crimescope MCS-400, SPEX Forensics) in Dissecting Microscopic Detection of Cryptic Epithelial Particles

ABSTRACT: As skin particles are often deposited by even a single skin contact, the detection of skin debris is important for evidence collection and DNA testing. Unfortunately, even when a dissecting microscope is used by an experienced examiner, epidermal particles are often hard to find and these traces of evidence might escape DNA analysis. Fluorescence is defined as the property of absorbing short-wavelength light and emitting longer-wavelength light. By virtue of the fluorescence characteristics of many target materials, tunable light sources assist in the macroscopic search of crime scenes and items. We combined the dissecting microscope and an alternate light source to examine the fluorescence characteristics of skin and skin particles. In a comparative study, small skin scales were hidden between sand, fibers, and soil probes, and it proved possible to search more successfully with less time and effort. On staged casework exhibits, the efficiency of the screening aid was again tested and the usability of the new procedure shown.

KEYWORDS: forensic science, forensic biology, tunable light source, skin debris, DNA

Fluorescence is the ability of a material to absorb light of a particular wavelength and then re-emit light at another, longer wavelength. A fluorescent substance may be excitable at one or maybe different wavelengths and may fluoresce at different wavelengths depending on the excitation wavelength used. Fluorescent characteristics of trace biological evidence were initially examined with the help of a so-called Wood's lamp an UV light source with a wavelength of around 350 nm. Special "forensic" light sources developed later, such as the Mini-Crimescope MCS-400 by SPEX Forensics (SPEX MCS-400 SPEX Forensics, Jobin Yvon Inc., Edison, NJ), are tunable. Via filters they only emit wavelengths in a narrow band and produce a specific emission spectrum. Therefore, the range of possible investigations was broadened, and previous research has demonstrated that an alternate light source can assist in the visualization and detection of biological stains, latent fingerprints, gunshot residues, etc. (1–5). It can also be helpful in dating the age of skeletal remains (6). On the other hand, it cannot yet assist in the identification of biological and other stains on skin (7) or help determine the age of a bruise (8). Regarding subsequent DNA analysis, it has been shown to not remarkably affect the results (9–11). Thus, the examination of exhibits with a forensic light source is a safe, simple, noninvasive and nondestructive screening aid to target areas of interest.

As a result of the desquamation process of the epidermis, we constantly dispense minute flakes of skin into the environment, which, for example, are deposited on touched objects, on pieces of clothing, or if we violently lay hand on someone. It has been shown that even a single skin contact, documented by a latent fingerprint, can transfer enough DNA for genetic analysis (12). Very often latent prints are not available, and the major problem in the use of skin particles is simply finding them. One procedure in such cases has been to cut out pieces or to take swabs from areas with a high possibility of containing these target particles and to set the sample directly into the DNA-extraction process. This technique is fast and generally accepted in most labs, but has distinct disadvantages. In order to reduce the possibility of missing evidence, often the maximum area is swapped or cut out. Without visual control, the target material is probably dispersed on the evidence instead of being assimilated. Therefore, in our experience, it is firstly far more preferable to seek cryptic epithelial particles with the help of a dissecting microscope, pick them up with a fine tweezers or a moistened needle and use them directly in DNA analysis. Because this is a very time-consuming approach that needs a very experienced examiner, we examined the possibility of aiding this procedure using a forensic light source.

Materials and Methods

Light Source

The alternate light source used in this study was a Mini-Crimescope MCS-400 by SPEX Forensics (SPEX MCS-400), which provides 16 different excitations ranging from 300 to 670 nm. The illuminant is a 400 W DAYMAX® metal halide lamp (SPEX Forensics) with a ceramic reflector, which has a larger light intensity than a 500 W Xenon bulb. The system includes a 2-m liquid light guide, 10-mm active diameter, and two remote filter wheels with eight positions each mounted on the working end of the light guide.

As mentioned above, the fluorescence must be observed at a wavelength greater than the incident excitation light, and therefore a filter is required to screen out any reflected incident or competing light. To achieve this filtering effect, a series of different-colored goggles was used. The shade of goggles worn (yellow, orange, red) becomes darker as the wavelength of incident excitation light is

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progressively shifted toward the longer end of the wavelength spectrum.

Dissecting Microscope

The dissecting microscope used (Carl Zeiss AG, Ober Kochen, Germany, OPMi I) delivers magnifications between 6× and 40× and allows documentation by a digital camera. It was necessary to carry out some modifications, before it was possible to observe fluorescence with the dissection microscope.

Using a specially built adaptor, the microscope was fitted with an optical filtering sliding hood that is usually placed on a digital camera within the SPEX Digi-Printscope for latent fingerprint documentation (Figs. 1*a* and 1*b*). The colored glasses in the sliding hood work as long-pass filters that allow light above a certain wavelength to pass through while filtering out light below that wavelength, just like the aforementioned goggles. Instead of using the light source of the dissecting microscope, the liquid light guide of the SPEX MCS-400 was fixed to the table by means of a large clamp so that it was possible to illuminate the exhibit from different directions.

In comparison to commercial fluorescence stereo microscopes such as the Olympus MVX, which offers a magnification between 4× and 250×, this home made system has advantages when working with forensic case material. Among these are a higher working distance, offering the opportunity to examine lager specimens, an extended field of view, and a richer variety of 16 different excitation wavelengths, which are purpose assembled for forensic issues.

Preliminary Testing

Studies on the fluorescence properties of skin have not been performed yet, but, there are publications concerning the detection of latent fingerprints (13–15), and the fluorescence of these is, besides sweat and fat, likely caused partially by skin particles.

We placed pieces of skin, collected from fingers and dandruff, on the adhesive side of transparent tape, which is used by the German police for evidence accumulation (HEROS asservation tape clear, Rosenbaum Company, Essen). The transparent tape was then folded with the adhesive sides against each other. Additionally, skin particles were lifted from the thenar eminence or the elbow and the transparent tape was folded in the same manner. With black cardboard as a background, the fluorescent characteristics were observed using excitation wavelengths ranging between 300 and 670 nm.

Test Series

Skin particles were hidden within four types of sand, fibers, and soil samples. For this, four to five scales of the outer skin or dandruff were placed on the adhesive side of transparent tape at positions marked with pinholes. The contaminant was added, and the transparent tape was then folded with the adhesive sides against each other. This procedure was repeated five times for each contaminant. Subsequently, one processor examined the pinhole-free side of the transparent tapes for the cryptic skin particles, using the dissecting microscope under natural light. Detected presumptive skin particles were marked with a pen, and after the examination, the success rates and time expenditures were recorded. The pen marks were then removed and the subsequent following processor repeated the examination using the SPEX MCS-400 light source. Finally, the success rates and the time expenditures were compared.

Additionally, we placed epidermal particles on different surfaces, such as textiles, stones, wooden shafts, and metals, and the visibility on these dummy casework exhibits was compared under natural light and with the alternate light source.

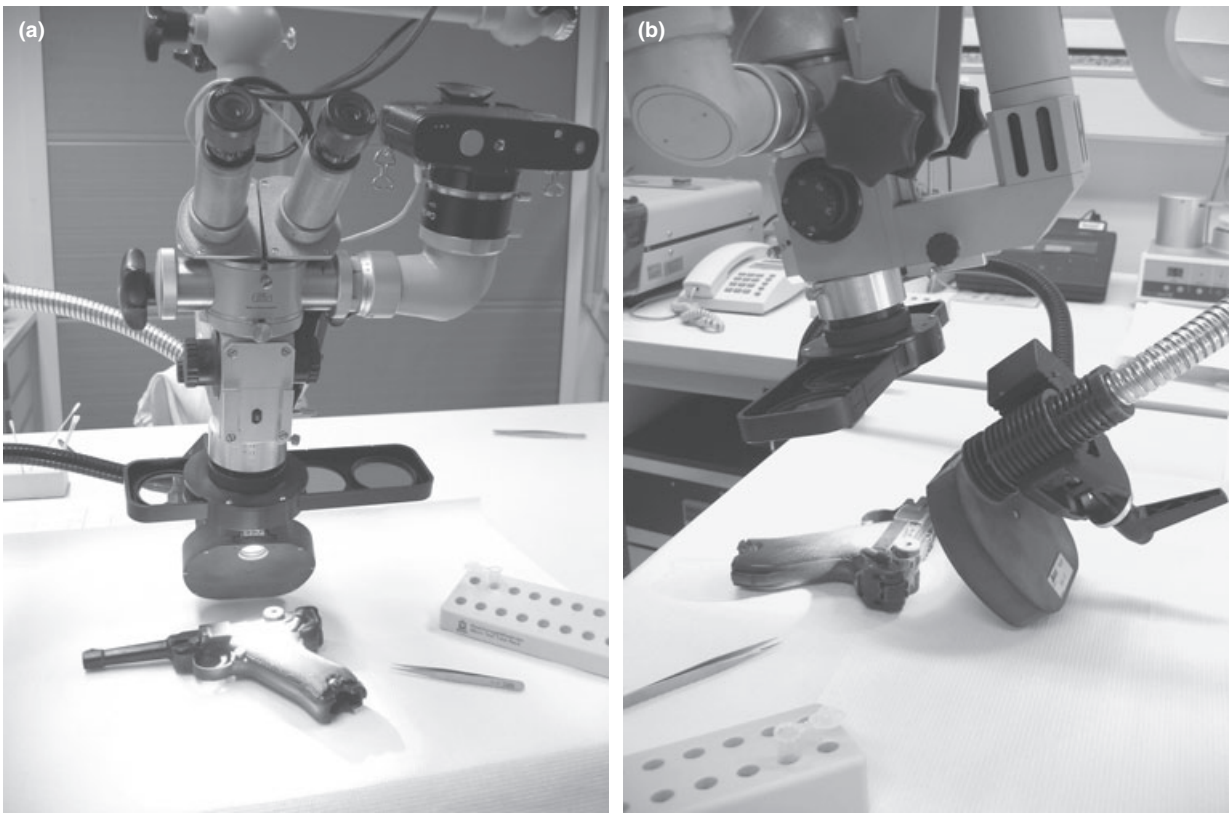

FIG. 1—*(a, b) The modified dissecting microscope.*

Results and Discussion

Preliminary Testing

We observed that fluorescence peaks can be best generated for skin particles at excitation wavelengths of about 390 and 475 nm (Figs. 2*b* and 2*c*). Nevertheless, these are simply orientation values and should be varied in order to minimize the effects of interfering substances. These substances can be the examined exhibit itself or a contaminant or both.

We found that irritations caused by reflections, scratches, or air bubbles and the glue of the transparent tape, which are visible under natural light (Fig. 2*a*), vanished when using the excitation light with the respective filters, and the skin particles became clearly visible and well defined (Figs. 2*b* and 2*c*).

Moreover, we observed that the strength of fluorescence of skin particles can vary. Somewhat oily and greasy dandruff particles, for example, seemingly emit more light than particles of dry skin, and, of course, fluorescence depends on the size and thickness of the skin flakes (data not shown).

Test Series

In the sand samples, we observed a dramatic enhancement in efficiency in the examination and detection of the skin particles. Under natural light, skin scales and little stones or quartz crystals are very hard to distinguish by size and color (Fig. 3*a*). Often a mistake is not realized until the putative skin scale bursts during preparation and reveals itself to be a small quartz plate. Using an excitation wavelength of about 390 nm or between 475 and about 510 nm, the skin and sand components could be discriminated quickly and easily (Fig. 3*b*). It proved possible to detect more skin particles in less than half the time (see Table 1).

With the fiber samples we found the situation to be different. If the epidermal scales were not totally buried, they were already visible under natural light (data not shown) and the search with the tunable light source simply became faster (see Table 1). The success rates and the benefit of the alternate illumination were influenced by the fluorescence characteristics of the examined fibers. At worst, these showed the same characteristics as the skin particles and the alternate light source was not a significant help.

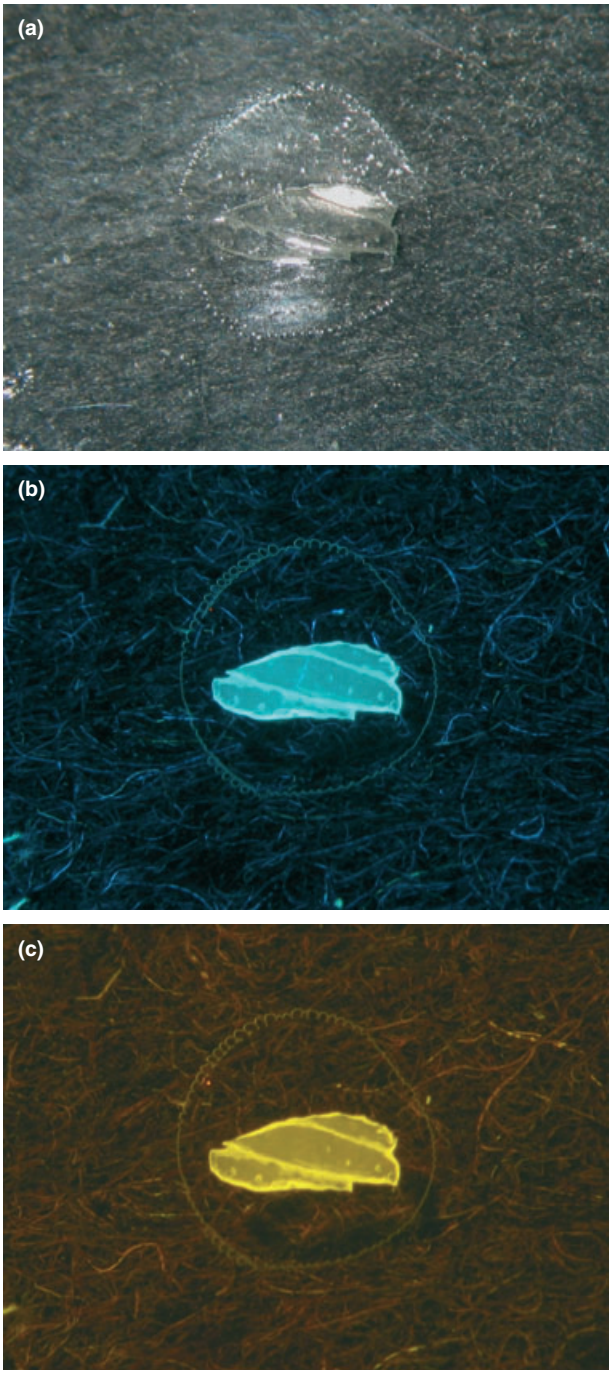

FIG. 2—*An approximately 2 × 1 mm piece of skin. (a) Natural light, (b) 390 nm, and (c) 475 nm excitation, viewed through a clear, yellow, and orange filter, respectively.*

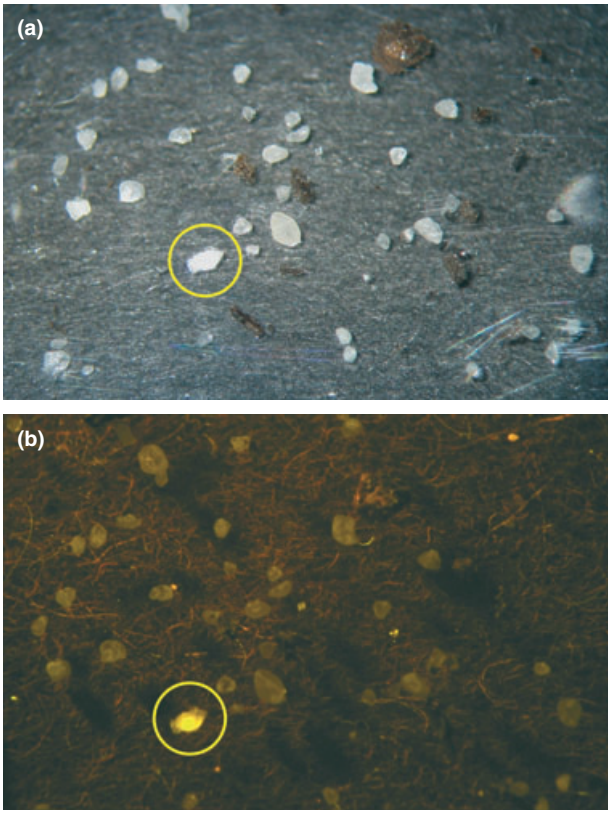

FIG. 3—*An approximately 1 mm^2 large scale of the outer skin (marked) hidden between sand particles. (a) Natural light and (b) 475 nm excitation, viewed through a clear and orange filter, respectively.*

TABLE 1—*Summary of skin particles hidden (H) and detected (D) within a time period (P).*

Samples	Total H	D (NL)	D (FL)	P (NL)	P (FL)
Sand	92	76	83	1 h 35 min	33 min
Fiber	95	64	60	57 min	38 min
Soil	90	39	64	1 h 42 min	62 min

NL, natural light; FL, forensic light.

Although some soil components showed a strong fluorescence, the examination became much easier using the tunable light source (see Table 1), because irritations, which can be ascribed to enclosed air or reflections or scratches on the transparent tape (Fig. 4*a*), were removed, and even epidermal particles covered with a thin coat of soil became visible (Fig. 4*b*). Nevertheless an experienced examiner is still essential to address unknown fluorescent particles as skin flakes or soil impurities.

The success with the staged casework exhibits strongly depended on the fluorescence characteristics of the examined item. Some textiles and lightly colored wooden handles caused a strong background fluorescence, which outshined the rather lightweight skin fluorescence (data not shown). In these cases, the alternate illumination could not improve the detection particles.

Another situation was found with dark wooden shafts. Under natural light, the epidermal particles were hard to find. Irritations were caused by reflections, contaminants, and crumbly or blistered clear varnish (Fig. 5*a*). With an excitation of 350 nm, the irritations were minimized, while the contrast increased and the visibility of the epidermal scales was improved (Fig. 5*b*).

The most impressive enhancement within the staged casework exhibits was obtained with stone material. By scratching the skin once, we created small areas of skin abrasion. On this, epidermal particles were initially not detectable, neither with the naked eye nor with the help of the dissecting microscope under natural light (Figs. 6*a* and 6*b*). First, the use of the tunable light source in combination with the dissecting microscope allowed the detection of skin particles (Fig. 6*c*).

Conclusion

In recapitulation, the combination of a tunable light source and a dissecting microscope can be a very helpful tool during the search for cryptic skin particles, depending on the fluorescence characteristics of the carrier or contaminating material. Sand and soil components are often almost indistinguishable from skin particles under natural light in terms of size and color. On wooden objects, epidermal particles and blistered clear varnish are difficult to distinguish and reflections additionally complicate the search. When collected with transparent tape, enclosed air and reflections hamper the identification of skin debris. In these cases, the use of the alternate light source and the filters can dramatically improve the detection and the epidermal particles become easily identifiable even when a variety of contaminants are present. On the other hand, some carrier materials and some impurities can fluoresce as well. It is known that soil components, fibers, and also laundry detergents (5)

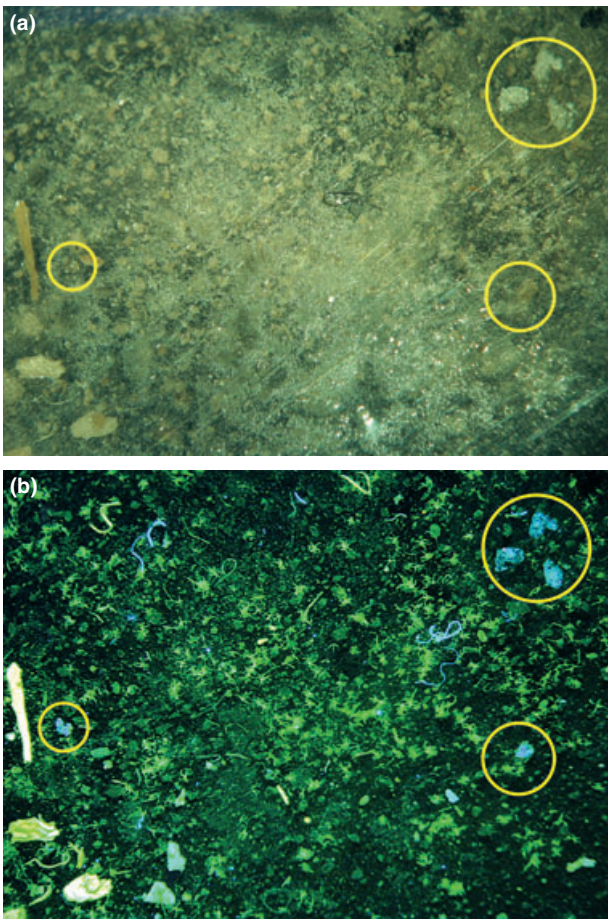

FIG. 4—*Five approximately 1 mm² large scales of the outer skin (marked) hidden between soil. (a) Natural light and (b) 390 nm excitation, viewed through a clear and yellow filter, respectively.*

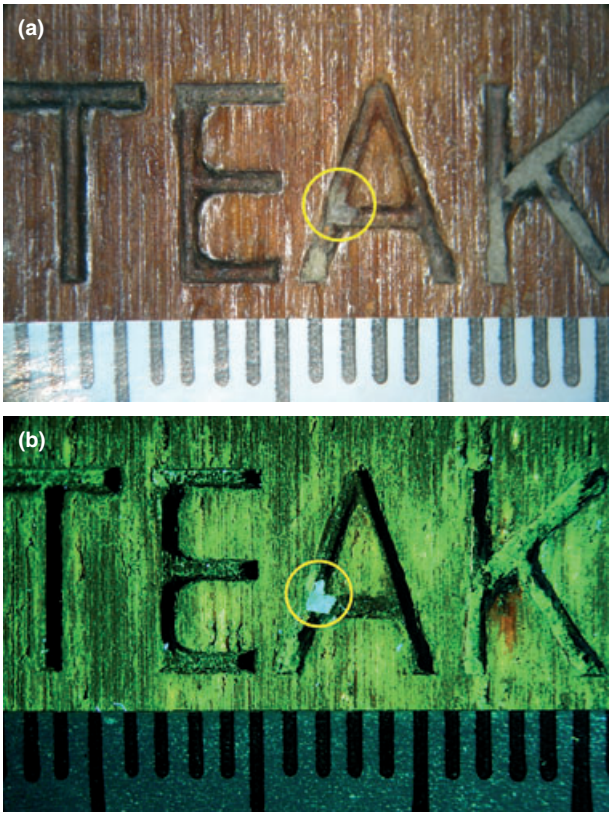

FIG. 5—*An approximately 1 mm² large scale of the outer skin (marked) hidden on a dark wooden handle. (a) Natural light and (b) 350 nm excitation, viewed through a clear and yellow filter, respectively.*

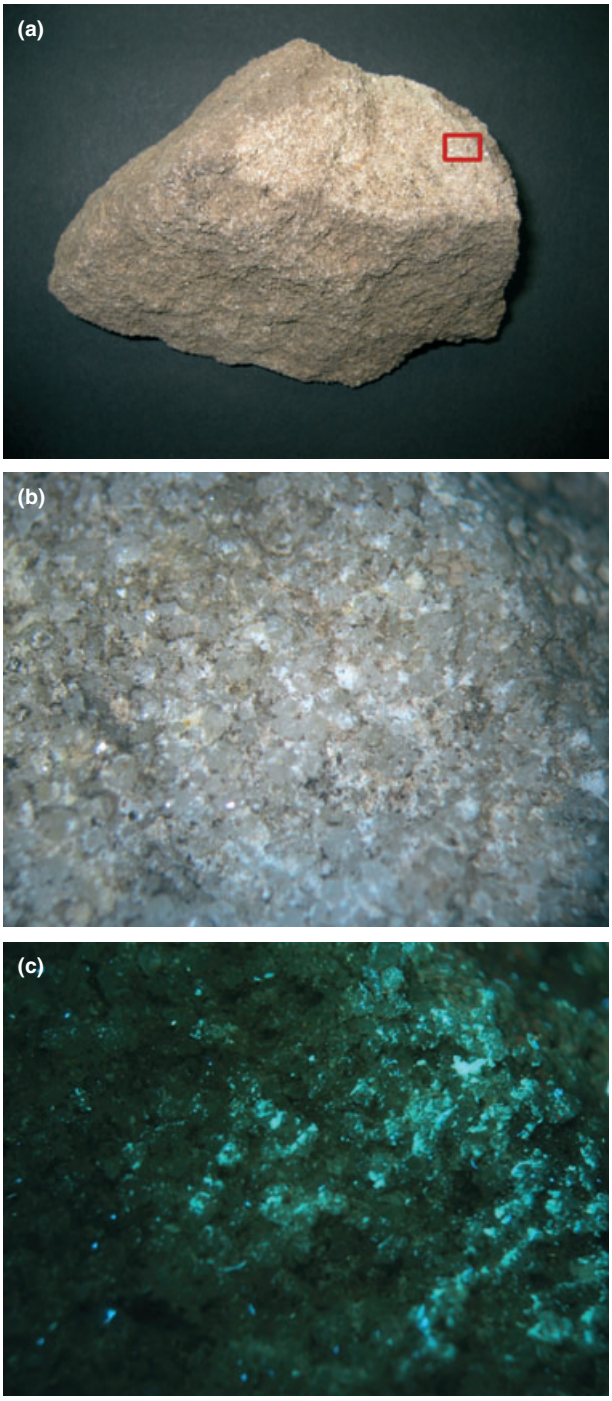

FIG. 6—*Stone with an approximately 12 × 10 mm large area containing skin abrasion. (a) Overview (area marked), (b) area under natural light, and (c) area at 390 nm excitation, viewed through a clear (b) and yellow (c) filter, respectively.*

can emit fluorescence. In some cases this background fluorescence may mimic the appearance of the skin particles and the application of the alternate light source is not indicated. Here, the use of a fluorescent dye such as DFO (1,8-diazafluoren-9-one), which reacts with amino acids, could be helpful by boosting skin fluorescence. While, up to the present, DFO has simply been used for the visualization of latent fingerprints (16), our experiences when using ninhydrin (2,2-dihydroxy-1,3-indandion), which reacts with amino acids as well, for the detection of skin particles are positive (17,18). The detection and selective removal of cryptic skin particles with the help of the tunable light source combined with the dissection microscope has become an inherent part of our procedures repertoire. However, even though the detection of skin particles has become more efficient, an experienced practitioner is still indispensable for the prevention of false-negative or false-positive results.

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