Microscopic imaging of human bloodstains: testing the potential of a confocal laser scanning microscope as an alternative to SEMs

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Abstract

The forensic interest on human bloodstains derives from their relation to crime investigation, whereas an archaeological and ethnographic concern arises from their occurrence because of warfare and ritual. The development of digital reflected light microscopes provided an opportunity to use light microscopy to study surface topographies in a more accurate way than previously. However, this enhancement has been focused on increasing magnification rather than resolution. An advanced type of light microscope is the confocal laser scanning microscope (CLSM). Its potential as an alternative to scanning electron microscopes (SEMs) for imaging human bloodstains was tested. A fragment of stone (brown chert) was smeared with human peripheral blood, air-dried, and stored indoors. After nearly two years, the sample was examined and imaged using an Olympus LEXT OLS4000 CLSM. The surface detail of CLSM images appeared to be comparatively lower than that of SEM micrographs of coated bloodstains taken at high-vacuum mode and high accelerating voltage, similar to that of SEM micrographs of uncoated bloodstains taken at low-vacuum mode and high accelerating voltage, and similar to or even higher than that of SEM micrographs of uncoated bloodstains taken at high-

vacuum mode and low accelerating voltage. These results suggest that a CLSM is a practical alternative to SEMs for imaging human bloodstains when a very-high level of surface detail is not required.

Keywords: blood smears, red blood cells, forensics, archaeology, ethnography, haemotaphonomy.

1. Introduction

Human bloodstains have a forensic, archaeological and ethnographic interest. The forensic interest on human bloodstains derives from their relation to crime investigation, whereas an archaeological and ethnographic concern arises from their occurrence because of warfare and ritual (e.g. Fiori, 1962; Torrence, 1993; Mazel et al., 2006).

The use of scanning electron microscopes (SEMs) is an efficient means of establishing the presence of blood. This is due to its high level of resolution for bulk objects, large depth of field, shadow-relief effect due to electron contrast, and ability to examine objects from very low to very high magnifications (Goldstein et al., 2003: pp. 1–2). Serviceable SEM micrographs of bloodstains can be obtained without coating the sample even via a high-vacuum SEM if working at very low accelerating voltage (Hortolà, 2005, 2008, 2013). However, usually 'environmental' or variable-pressure SEMs working in low-vacuum mode and high accelerating voltage are employed when examining uncoated samples (e.g. Hortolà, 2012, 2016).

When on relatively large objects such as household items or white weapons, bloodstains cannot be directly examined with a conventional SEM because the specimen does not fit into the sample chamber. This problem can be avoided using an unconventional, large-chamber SEM or, more commonly, synthetic replicas (e.g. Klein and Brandt, 2006; Huq et al., 2008; Hortolà, 2015). Large-chamber SEMs are remarkably expensive and, therefore, unusual among the microscopy facilities offered by most universities and research centres. On the other hand, the disadvantage of synthetic replicas addressed to the study of surface topographies is that they are time-consuming in making.

Unlike conventional SEMs, light microscopes avoid the sample size constraint to a large extent. Furthermore, new developments in light microscopy have provided an opportunity to study surface topographies in a more

accurate way than previously. An advanced type of light microscope is the confocal laser scanning microscope (CLSM), which plays a similar role to SEMs in materials science (Hovis and Heuer, 2010). The fundamentals and genesis of CLSMs can be found elsewhere (e.g. Kaplonek and Nadolny, 2012; Murphy and Davidson, 2013: pp. 265–305).

Previously, I have reported the use of SEMs to examine and image human bloodstains (e.g. Hortolà, 1992, 2002, 2012, 2016). In this paper, I report the use of a CLSM to test its potential for imaging this kind of material, as an alternative to that electron microscope.

2. Materials and methods

A fragment of stone (brown chert) was smeared with a very small volume of human peripheral blood from a hand's palm by the mechanism of 'contact'. Subsequently, the blood was air-dried indoors at a room temperature of 22 °C. After indoor storage for nearly two years (22 months), the sample was examined and imaged using an LEXT OLS4000 CLSM (Olympus Corp., Tokyo, Japan).

3. Results and discussion

CLSM micrographs, arranged at increasing magnifications, are exhibited in Figs. 1–3. Fig. 1 displays a partial view of the bloodstain-substrate boundary. In Fig. 2, erythrocytes out of the periphery, towards the central area of the bloodstain, can be seen. Fig. 3 depicts a detail of the assemblage of erythrocytes observable at the surface of the bloodstain.

At first sight, the surface detail of CLSM images is comparatively lower than that of SEM micrographs of coated bloodstains taken at high-vacuum mode and high accelerating voltage (compare, for example, with Hortolà, 1992). However, such detail is similar to that of SEM micrographs of uncoated bloodstains taken at low-vacuum mode and high accelerating voltage (compare, for example, with Hortolà, 2016). Finally, the surface detail is similar to or even higher than that of SEM micrographs of uncoated bloodstains taken at high-vacuum mode and low accelerating voltage (compare, for example, with Hortolà, 2005).

In the test reported here, a sole small smear was used because the erythrocyte count in whole blood is very high. This applies to all the vertebrate classes and especially to mammals (several authors, in Ragan et al.,

2003: Tables B.1. − B.2.). A reference range for the erythrocyte count in adult humans is 4.5–5.7 million per microlitre in males and 4.0–5.3 million per microlitre in females (University of Kentucky Chandler Medical Center, 2006: pp. 54–55).

Obviously, to analyse the micromorphology of any sort of smear does not depend on the type of microscope provided that it be suitable for visualising surfaces (i.e. with incident electron beam or with visible light). However, using a CLSM forensic scientists, archaeologists or ethnographers could easily gather evidence in putative bloodstains using an enhanced light microscopy technique that can be used as a screening method preventing possible damage of the sample due to the electron beam of SEMs.

Surface topography analysis of a bloodstain is not appropriate for doing a blood typing or a DNA matching, nor does it allow discriminating taxonomically beyond mammalian vs. non-mammalian blood. However, such analysis is important in a forensic, archaeological or ethnographic context. A reason is that to screen the presence of blood using a nondestructive technique such as the used in this work, allows further analyses to be performed via other techniques, avoiding to damage unnecessarily valuable smears and/or their substrates (trial pieces of evidence, museum specimens). Other reason, specific to human forensics, is that the microscopical examination of a bloodstain can assist to restrict the target individual in case of victim/suspect's blood cell disease such as sickle cell anemia or ovalocytosis. In conclusion, the results of this test suggest that a CLSM is a practical alternative to SEMs for imaging human bloodstains when a very-high level of surface detail is not required. Noticeably, further work is essential for establishing the best ad-hoc CLSM working conditions. Finally, it is worth pointing out that, while this work is centred on blood smears, it is well-probably applicable to other types of samples. And, accordingly, it can also be relevant to people engaged in many science and engineering fields, from microscopic anatomy to materials

science.

Declaration of Competing Interest

The author declares no competing interest.

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Figures

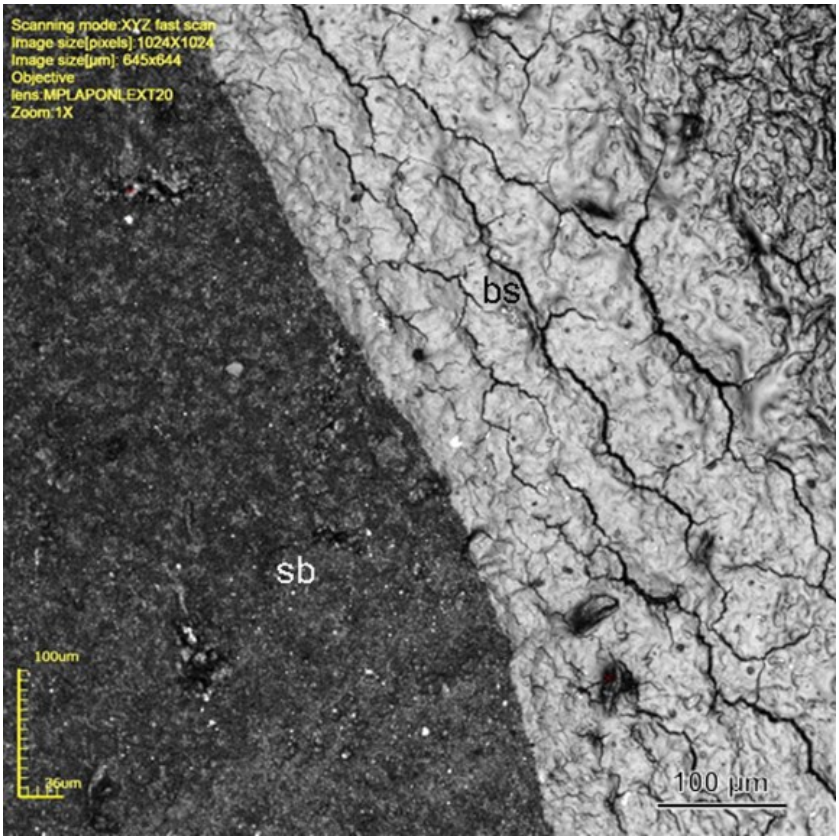

Fig. 1. Partial view of the boundary between the bloodstain (bs) and its substrate (sb), at low magnification. For viewing comfort, a customary scale bar has been added. CLSM micrograph original magnification: 279×.

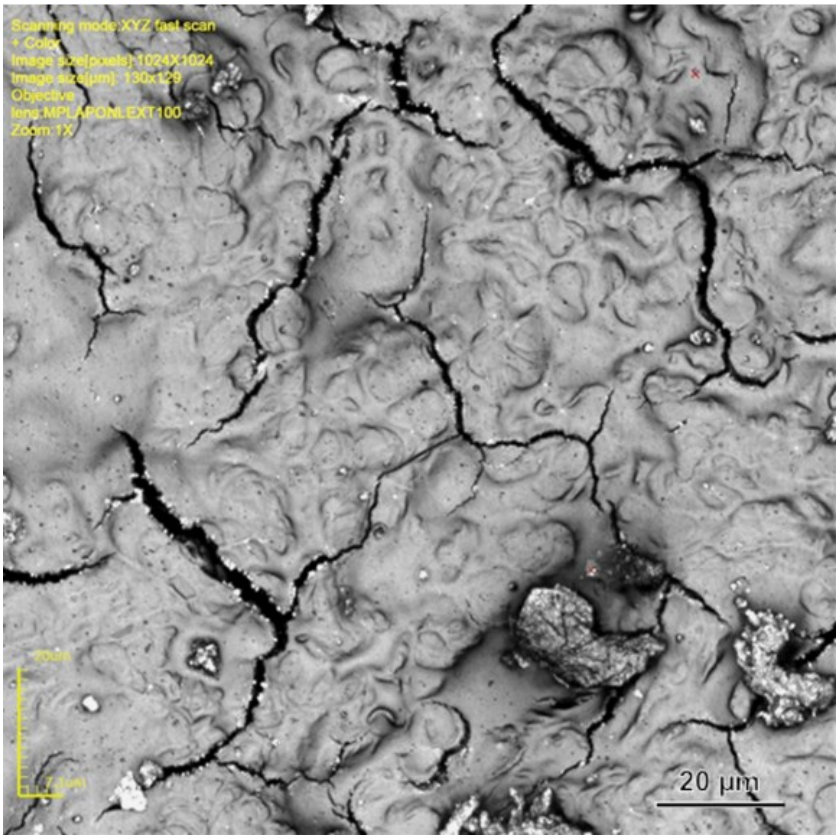

Fig. 2. Erythrocytes out of the boundary, towards the central area of the bloodstain, at medium magnification. The flamboyant bright particles are dust settled *a posteriori* on the smear. For viewing comfort, a customary scale bar has been added. CLSM micrograph original magnification: 1390×.

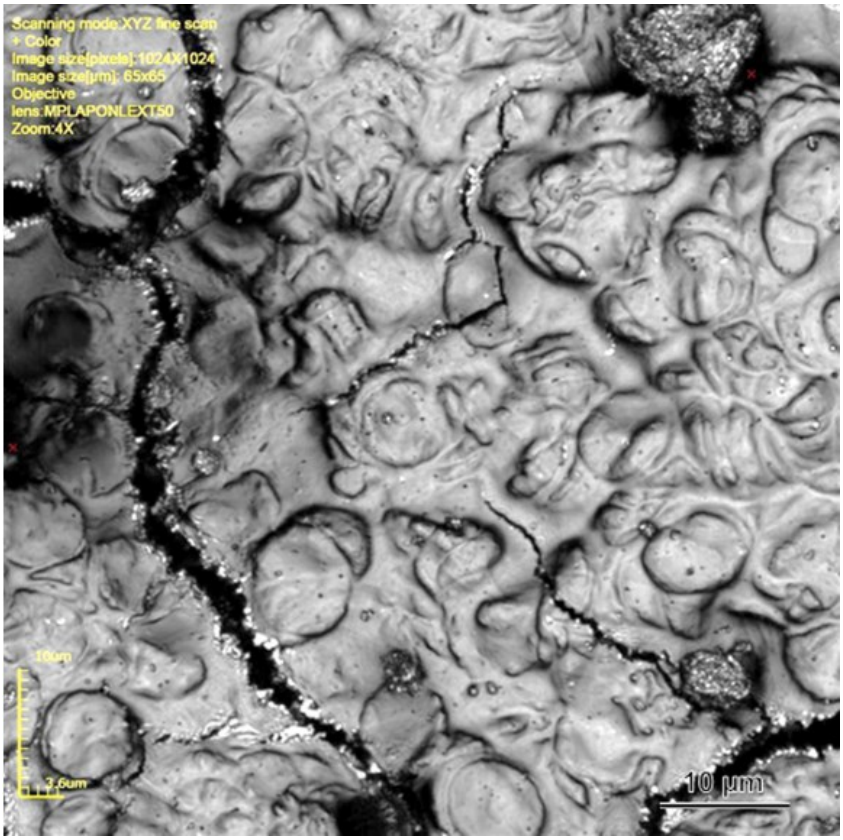

Fig. 3. A detail of the assemblage of erythrocytes observable at the bloodstain surface, at high magnification. The flamboyant bright particles are dust settled *a posteriori* on the smear. For viewing comfort, a customary scale bar has been added. CLSM micrograph original magnification: 2800×.