

Visualisation by confocal microscopy of traces on bullets and cartridge cases

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The capabilities of confocal scanning laser microscopy for the visualisation of marks on bullets and cartridge cases were investigated. Confocal microscopy provides solutions to important limitations of conventional comparison microscopy with grazing light incidence, as generally used for the examination of these marks. It is expected that confocal microscopy, thanks to its broad applicability within the field of firearms investigation and its capability of non-destructively gathering quantitative three-dimensional information, will lead to a more complete and objective forensic examination of bullets and cartridge cases.

La capacité de la microscopie laser confocale à balayage pour l'observation de marques sur les balles et les douilles a été étudiée. La microscopie confocale donne des solutions aux limitations importantes de la comparaison microscopique conventionnelle à l'aide d'une lumière incidente rasante, telle qu'elle est généralement pratiquée dans l'examen de telles marques. Il est attendu que la microscopie confocale, à cause de son applicabilité étendue dans le domaine de l'investigation des armes à feu et sa capacité d'obtenir une information quantitative à trois dimensions, conduira à un examen plus complet et objectif des traces sur les balles et les douilles.

Es wurden die Fähigkeiten der konfokalen Scanning Laser Mikroskopie für die Visualisierung von Verfeuerungsspuren auf Kugeln und Patronen untersucht. Die konfokale Mikroskopie stellt Lösungen für die gravierenden Einschränkungen der konventionellen Vergleichsmikroskopie mit Seitenbeleuchtung zur Verfügung, die üblicherweise für die Untersuchung solcher Spuren eingesetzt wird. Es wird erwartet, dass die konfokale Mikroskopie, dank ihrer breiten Einsatzfähigkeit auf dem Gebiet der Waffenuntersuchung und dank ihrer Fähigkeit zerstörungsfrei dreidimensionale Bildinformationen zu liefern, zu vollständigeren und objektiveren forensischen Untersuchungen von Kugeln und Patronen führt.

Se ha estudiado la capacidad de la microscopía láser confocal de barrido para la observación de marcas sobre balas y casquillos. La microscopía confocal aporta soluciones a las importantes limitaciones de la comparación microscópica convencional, con la ayuda de una luz incidente rasante tal como se usa generalmente para el examen de estas marcas. Es de esperar que la microscopía confocal, en virtud de su extendida aplicabilidad en la investigación de las armas de fuego y su capacidad para recoger cuantitativamente de manera no destructiva información tridimensional, conducirá a un examen forense de balas y cartuchos más completo y objetivo.

Introduction

The standard procedure employed by firearms examiners in comparing characteristic marks on bullets and cartridge cases has not changed significantly for several decades. The primary examination technique involves the simultaneous examination of the evidence mark and a test mark using a split-field conventional comparison microscope with incident light illumination [1–9]. This observation technique employs two nearly independent microscopes, complete with separate positioning and orientation controls for each stage, focusing and other optical components, to present the images of both objects to the two halves of the field of view of one eyepiece [4,7]. The images generated with this technique give a representation of the surface of the mark. This representation is critically dependent on a number of parameters, such as the substrate in which the mark has been made, the geometry and intensity of the illumination source, the position of the sample, etc. [3,10–13]. It frequently happens that the characteristic features cannot be viewed at the same time, e.g. the illumination on one area should come from its left side, while on an adjacent area a right-hand source is needed [4]. Changes in illumination can thus make some features stand out clearly, but at the expense of others perhaps equally prominent [4,10,14]. In addition, with conventional comparison microscopy, only a two-dimensional (2D) image of the specimen can be formed. This means that the original three-dimensional (3D) structure of the specimen, which may hold important information for the comparison of two different specimens, is not accessible. Therefore, the question whether or not two specimens are of common origin can often not be answered to full satisfaction [7].

Specific techniques employed in examinations made with comparison microscopy have changed little since this instrument was first developed [2]. Whereas variations and improvements in microscopes, lenses, cameras and light sources have occurred over the years, the underlying techniques have seldom been altered to any extent [2,15]. It should be highlighted that some efforts have been made to introduce new methods and to optimise known techniques. However, many unsolved problems still persist in this particular field of firearm identification [16].

Aim of the study

The aim of this work is to determine the usefulness of confocal scanning laser microscopy in the firearms identification. Confocal microscopy is a form of optical microscopy which is capable of visualising the 3D structure of microscopic objects. While testing a new technique, one should keep in mind the drawbacks which affect the visualisation and analysis of class characteristics (such as the width and the rate of twist of the land impressions, the width of the firing pin impression, etc.) and individual characteristics (such as the striations left by the lands of the barrel, the breech face, the firing pin, etc.) by means of the conventional optical methods. In this article we will summarise the main drawbacks of current conventional optical methods and indicate whether confocal microscopy gives better or worse results.

Principles of confocal microscopy

The optical layout of a confocal microscope is shown schematically in Figure 1. Generally a laser is used as the illumination source. With the help of an auxiliary lens the laser light is focused onto an illumination pinhole with a typical diameter of 50 to 100 micron. With this construction, a so-called 'point light source' is created. The light transmitted through the pinhole is sent onto a beam splitter, which reflects 50% of the incident light to the objective lens, which in turn focuses the light to a small illumination spot in its focal plane. The spatial dimensions of the illumination spot determine the resolution of the microscope: for state of the art objective lenses, the lateral and axial resolutions are respectively 250 nm and 700 nm. The light reflected by the object is collected with the objective lens, is transmitted (again for 50%) by the beam splitter and is focused onto the detection pinhole. Essential for confocal microscopy is that the distance between the illumination pinhole and the objective lens is equal to that of the detection pinhole and the objective lens. Light reflected from the focal plane passes freely the detection pinhole, whereas light reflected from planes below or above the focal plane is focused below and above the detection pinhole and is thus effectively blocked by the pinhole. In this way, in-focus information is separated from out-of-focus information. The in-focus reflected light is registered with the photodetector. In standard confocal microscopes a photomultiplier tube (PMT) is used for signal detection.

Although this set-up is capable of discriminating between in- and out-of-focus light, no image is formed at this stage. Image formation is accomplished by scanning the illumina-

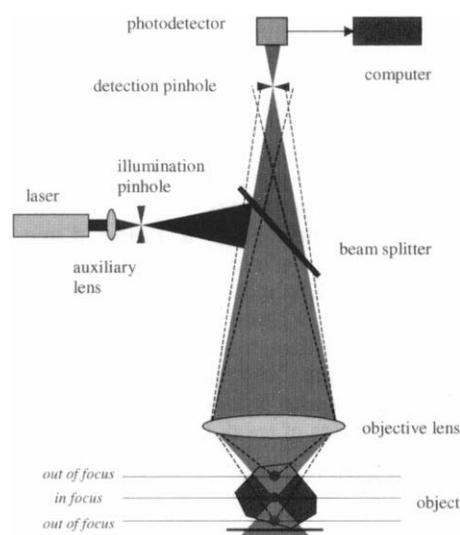


FIGURE 1 Schematic layout of a confocal microscope.

tion spot (scanning unit not shown in Figure 1) over the specimen, measuring the intensity of the in-focus reflected light with the photodetector and storing the signal in computer memory. In this way, a two-dimensional confocal image (also referred to as an 'optical section') containing

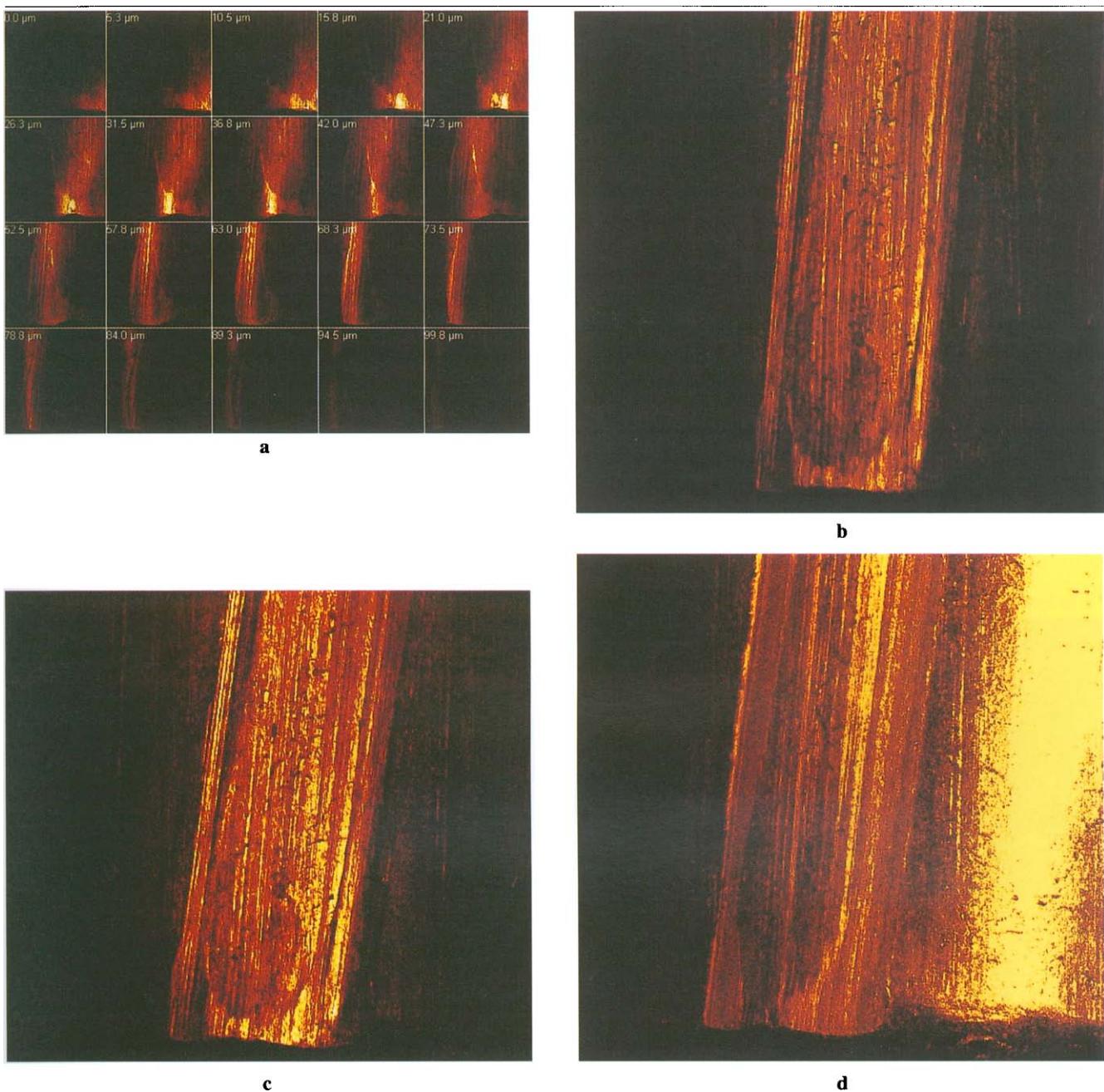


FIGURE 2 Confocal images of a land impression on bullet B1. (a) 3D confocal image visualised using an image gallery. In each image different parts of the mark are in focus (white) and out of focus (grey and black). The numbers in the upper left corner represent the axial position relative to an arbitrarily chosen reference plane. Objective lens: 10x, image dimensions: 0.9x0.9x0.1 mm. (b) Extended focus projection of a 3D confocal image. (c) Extended focus projection of the mark visualised in (b), after repositioning of the bullet. (d) Extended focus projection of the mark visualised in (b), after deliberate mispositioning of the bullet. For (b), (c) and (d), a 2.5x objective lens was used; image dimensions: 3.6x3.6x0.7 mm.

only information about that part of the object in the focal plane of the objective lens, is formed. A 3D image is obtained by acquiring a set of 2D confocal images while translating the object after each 2D image towards the objective lens. Provided that the separation between the individual 2D images is chosen correctly, the resulting stack of optical sections contains the complete 3D information of the object.

It should be noted that besides confocal reflection

microscopy described here, confocal microscopy can also be used in fluorescence and (although not common) in transmission modes. More information about confocal microscopy can be found in 'The Handbook of Biological Confocal Microscopy' [17].

Data visualisation techniques

To fully exploit the capabilities of confocal microscopy, the 3D data must be visualised on a 2D surface (a photograph or a laser print) in such a way that the 3D structure of the

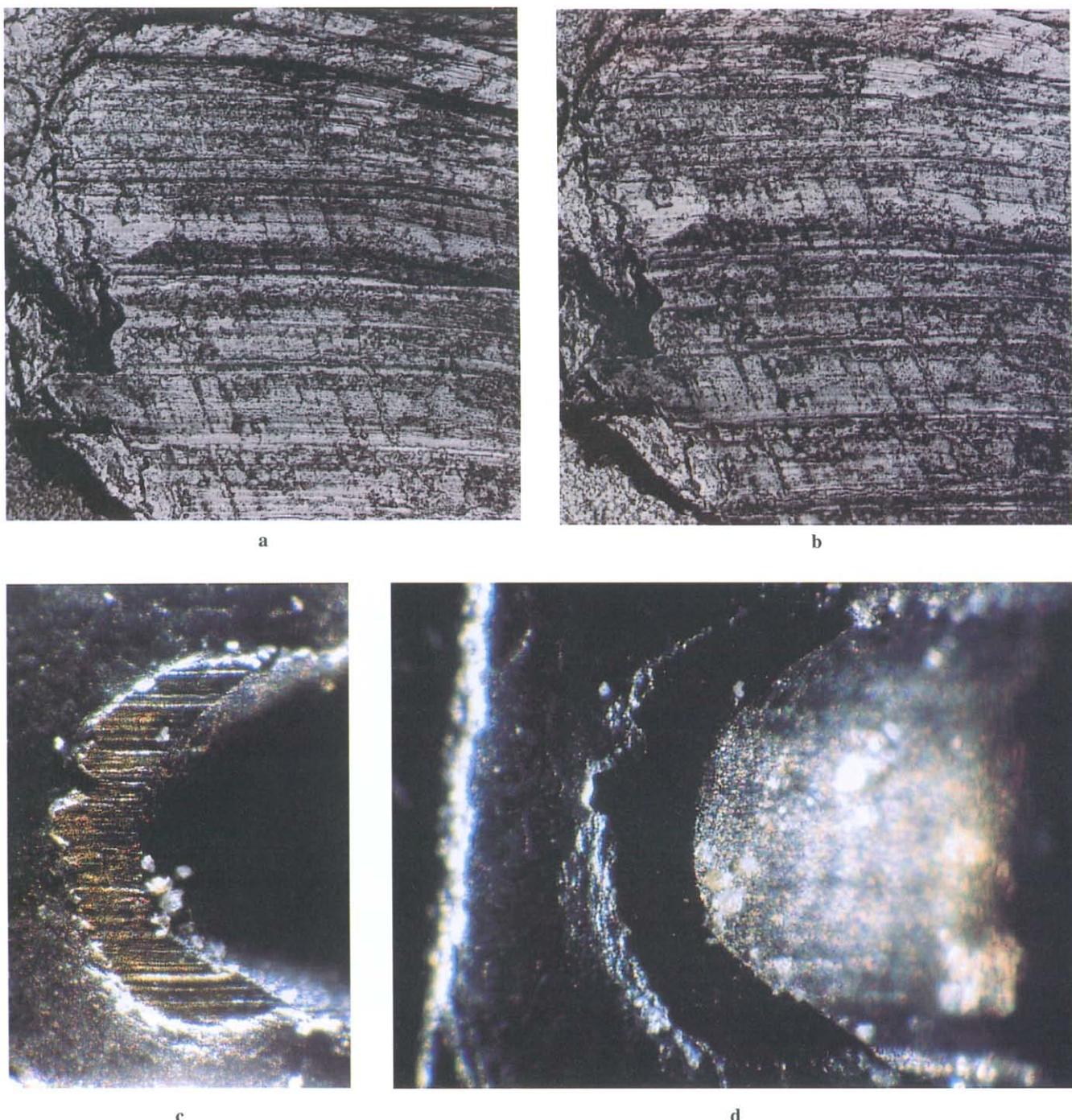


FIGURE 3 Extended focus projections of a 3D confocal image of the breech face mark of cartridge case C1. (a) First projection. (b) Projection after rotating the cartridge case 90 degrees clockwise. For viewing convenience, the projection was rotated 90 degrees counter clockwise. For both (a) and (b), a 50x objective lens was used; image dimensions: 185x185x26 micron. (c) and (d) Conventional pictures of the marks shown in (a) and (b). A 50x objective lens was used; image dimensions: 0.9x0.7x0.1 mm.

imaged object is expressed. Many types of 3D visualisation are available: from simple galleries of all confocal images to complex 3D surface visualisation schemes. In galleries, all single optical sections are displayed in a table. The differences and similarities between the separate images give basic information of the 3D structure of the object (see Figure 2a). Instead of displaying the single optical sections separately, one can also sum the intensities in the separate

images pixel by pixel. This data visualisation method is referred to as an 'extended focus projection', since all the different depths imaged in the 3D confocal image are visible, with equal contrast and detail, in a single 2D image (Figure 2b).

A more advanced visualisation technique are the so-called orthogonal sections (also referred to as 'optical cross sections'). Designating the plane of the optical sections as the

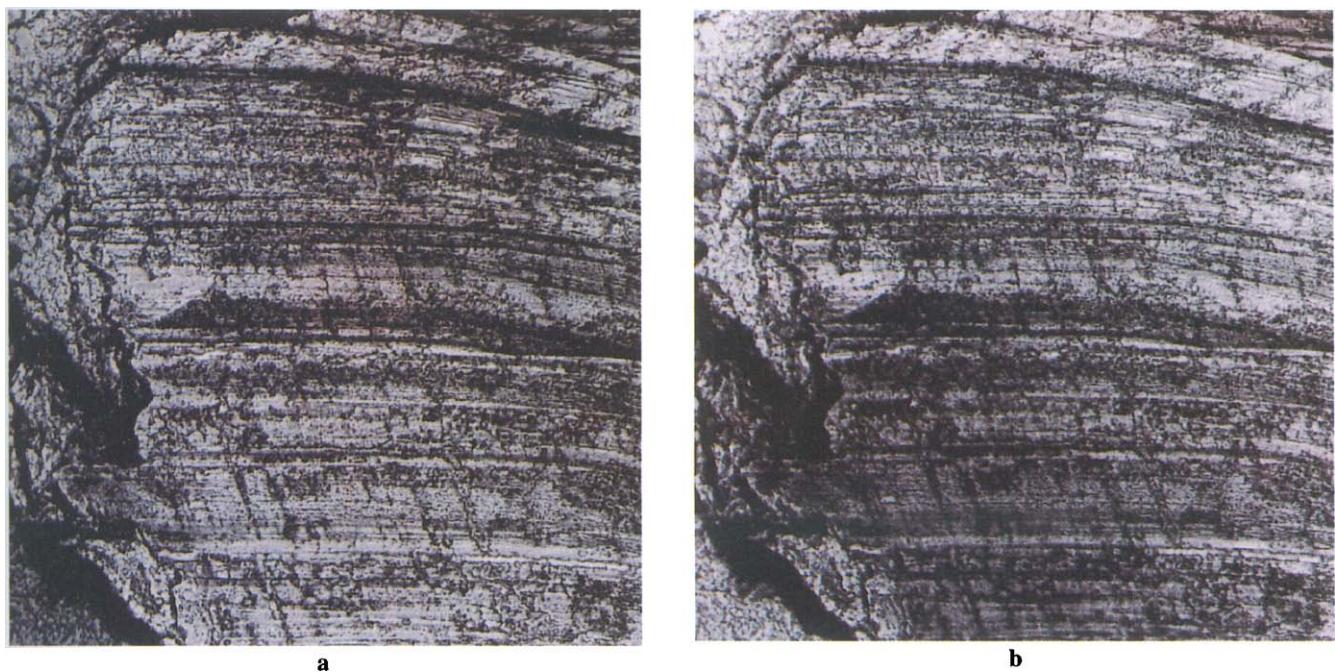


FIGURE 4 Extended focus projection of a 3D confocal image of the breech face mark of cartridge case C1. (a) Acquired at a low laser intensity. (b) Projection of the image of the same segment shown in (a), acquired at a high laser intensity. For both (a) and (b), a 50x objective lens was used; image dimensions: 185x185x26 micron.

xy-plane and the direction perpendicular to the optical sections as the z-axis, orthogonal sections, as calculated from the full 3D data set, display the yz- and xz-views (respectively the side- and front-views) of the objects in the data set (see Figure 6b).

Even more advanced is the so-called 'surface rendering' technique, a 3D data visualisation method which highlights the surface of the objects in the 3D data set. Surface segments are assigned to rapid intensity changes, detected through so-called 'gradient methods' (see Figures 6c and 7).

For information on other types of 3D data visualisation methods, the reader is referred to 'The Handbook of Biological Confocal Microscopy' [17] and to 'Computer Graphics: Principles and Practice' [18].

Materials and methods

Specimens

Two different kinds of specimens were used: i) bullets and cartridge cases and ii) casts of cartridge cases. Bullets were fired in a 9 mm Luger semi-automatic Pietro Beretta 92 S pistol (referred to in the text as B1), whereas cartridge cases were fired in two different 9 mm Luger semi-automatic Glock 19 pistols (denoted in the text as C1 and C2); both the bullets and cartridge cases were fixed on standard microscope slides using double sided adhesive tape.

For the casts, the following three casting materials were used: Xantopren® VL Plus with Optosil®-Xantopren® Activator (Bayer Dental, Leverkusen, Germany), Sta-Seal with catp universal (Detax, Ettlingen, Germany) and Silmark low viscosity grey (BVDA International, Amsterdam, The Netherlands) with Mikrosil Hardener

(Kjell Carlsson Innovation, Sundbyberg, Sweden). The casts were directly prepared on microscope slides. Only the casts of cartridge case C1 were measured.

Confocal microscope

A Zeiss Axioplan 2 microscope equipped with a confocal scanning unit (LSM 510, Carl Zeiss, Germany) and an air-cooled Argon-Krypton ion laser (emitting at 488 nm and 568 nm, Omnicrome, U.S.A.) was used. This system forms the hardware basis of the project 'Forensic Applications of Confocal Microscopy', as carried out at the Netherlands Forensic Science Laboratory. Images were acquired with 2.5x, 10x and 50x air lenses and a 40x oil immersion lens, in the confocal reflection mode. For all images the excitation wavelength was 488 nm and a standard photomultiplier tube was used for signal detection. Microscope control and image collection (using the software package 'LSM 510' (Carl Zeiss)) were done with an IBM compatible computer. Visualisation was performed using the same software package as well as '3D for LSM' (Carl Zeiss).

When using air lenses, the specimens were viewed without any further preparation. For the oil immersion lens, the specimens were covered with a glass coverslip with standard immersion oil between the specimen and the coverslip and between the coverslip and the objective lens.

For each specimen, stacks of optical sections (each stack constituting one 3D image) were acquired. Each optical section consisted of 512x512 pixels (dimensions chosen arbitrarily). The number of optical sections within one stack was depending on the ratio between the depth of the fea-

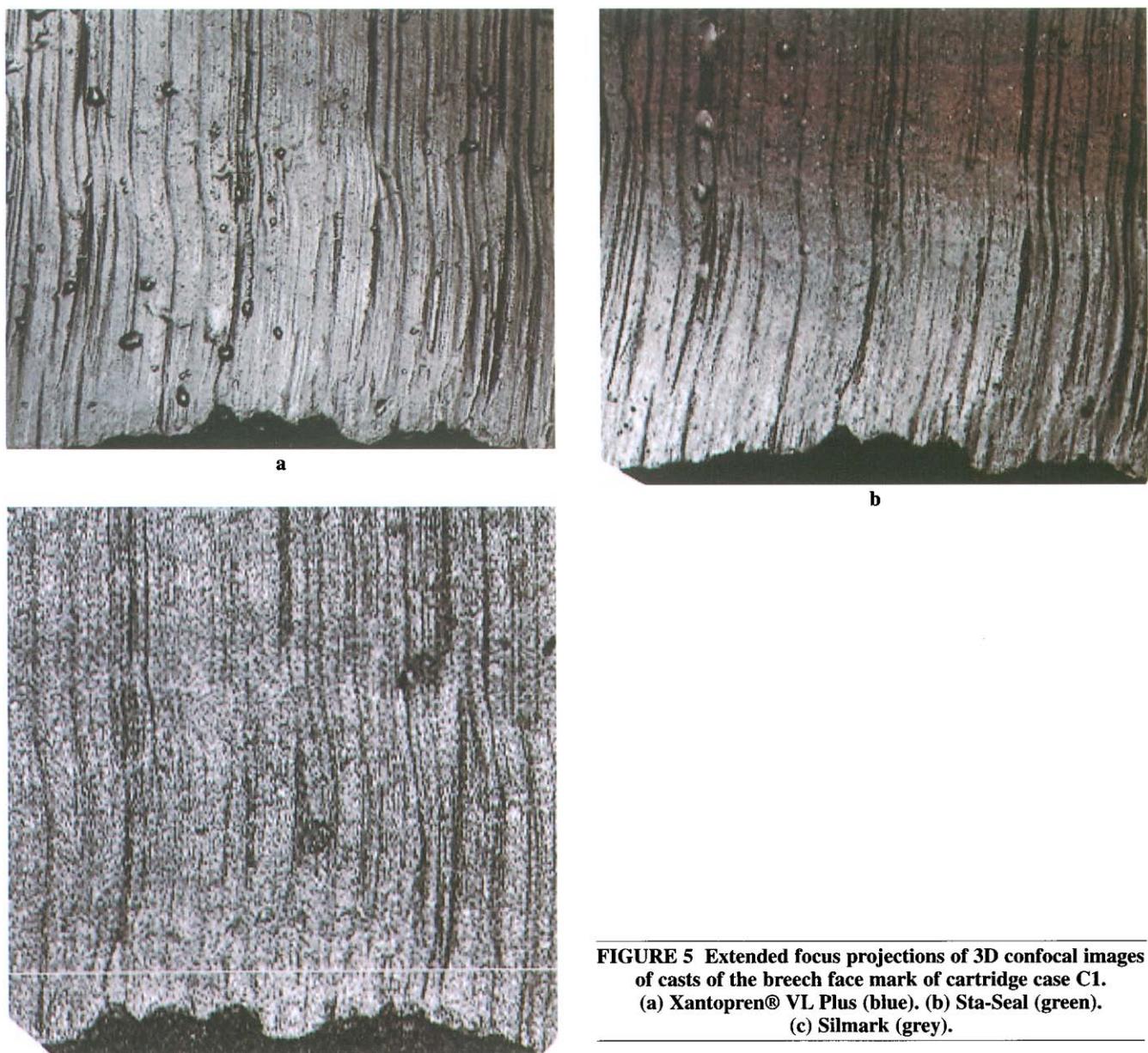


FIGURE 5 Extended focus projections of 3D confocal images of casts of the breech face mark of cartridge case C1.
(a) Xantopren® VL Plus (blue). (b) Sta-Seal (green).
(c) Silmark (grey).

tures in the specimen and the spacing between the optical sections. For all 3D images, the section spacing was set at the optimum spacing for the specific lens used (see also Pawley [17] for more details about optimum section spacing and objective lens properties). Depending on the number of sections within the 3D image, acquisition of a 3D confocal image requires one to five minutes.

Conventional microscope

In some cases, we imaged the traces visualised in the confocal mode using conventional microscopy by means of a Leica Wild M420 equipped with two opposite sidelights. When discussing phenomena well known to firearms examiners, we omitted the conventional pictures for brevity. One has to take into account that when using the same magnification lenses in both microscopes, the fields of view may be different (see Figures 3a–3d).

Problems encountered in using conventional comparison microscopy to observe bullets and cartridge cases and possible solutions when using confocal microscopy

1. The geometry of the light sources and the incident angle of the light beam with respect to the bullets/cartridge cases

A. Conventional microscopy

As stated before, the visual examination and comparison with the aid of various low magnification (10x to 30x) light microscopes is currently the most widely accepted and used method for the routine comparison of class and individual characteristics [19,20]. Practically, the examiner applies lighting at various angles and intensities to highlight small elevations of the surface [3,4,9,21–23]. An important complication in this approach, however, is that small changes in the angle of the incident light can have an appreciable effect on the image [4,16,22,24–26]. Actually, the observed traces

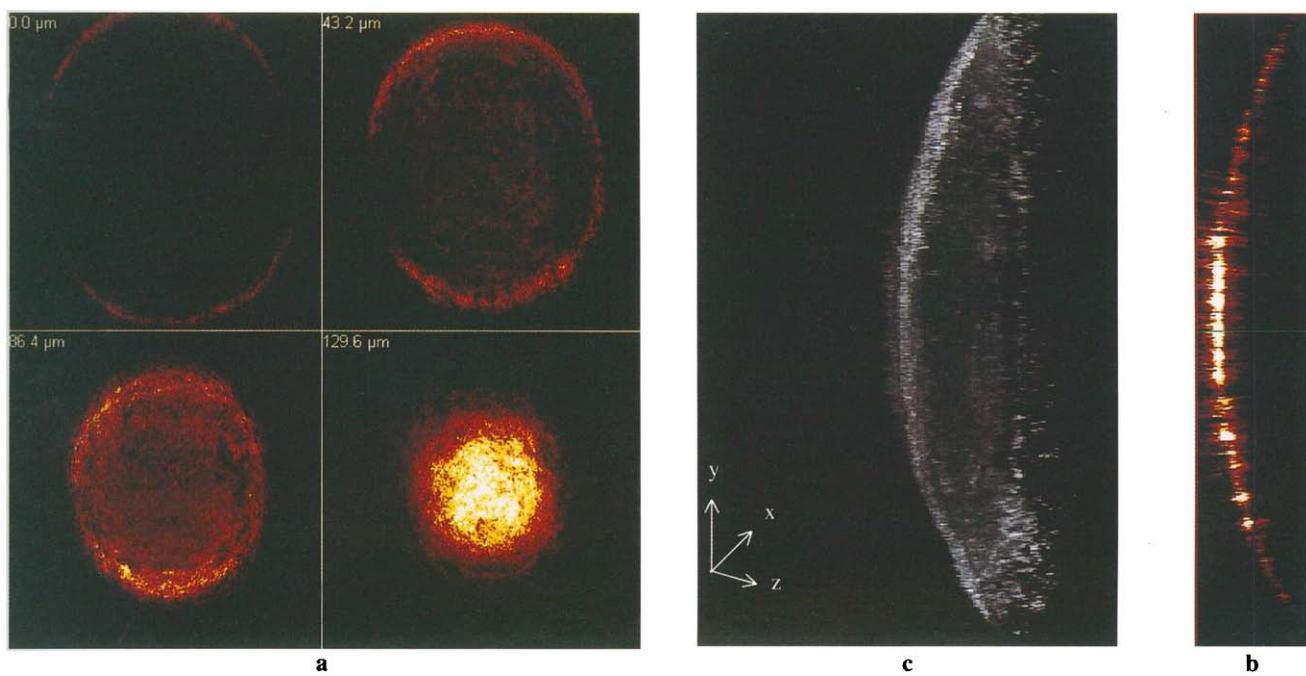


FIGURE 6 Confocal images of the bottom of the firing pin impression of cartridge case C2. (a) Single confocal sections (four selected from the complete confocal data set), acquired at different depths, showing the ellipticity of the impression. (b) Orthogonal section as calculated from the full confocal data set. (c) Rotated surface rendering of the confocal data set, showing the full 3D shape of the firing pin impression bottom. For (a), (b) and (c), a 10x objective lens was used; image dimensions: 0.9x0.9x0.1 mm.

will appear as a series of narrow light and dark strips and, therefore, the third dimension is inferred by shadowing [9,20,27–29]. The width and the depth of the shadows are determined by the illumination conditions [10,11,30]. Unless the illuminations are at the same angle, the details revealed by the brightness and the shadows will not be alike and can confuse the examiner [10,13,29]. According to several authors, it is almost impossible to illuminate two objects in the same way and therefore to create the same ‘shadow effect’ [15,27,31]. The shadows provide fictitious values to the depressions and indentations found on the objects [30]. A particular consequence of this phenomenon is the ‘Azimuth Effect’ [32], which can be seen by observing the two edges of a material cut or sliced with a tool. These marks can be viewed as opposites under parallel lighting or mirror images under opposed (reverse) lighting [33–36]. Since the appearance of the traces is different using different angles of illumination, the firearm examiner may confirm a match using illumination from more than one angle [10].

Three ways of lighting can be envisaged, which will be discussed in this paper later on: i) oblique lighting, ii) vertical (ring lighting) and iii) co-axial lighting. i) Most examiners apply oblique lighting and they recommend an incident angle of 45 degrees with the plane of the marks. When the lamps are set at an angle approaching grazing incidence to the marks much greater contrast is produced [14,37,38], but the surfaces to be observed are often unevenly illuminated. For striated marks, the light beams are directed at right angles to the length of the striated marks [4,9,11,

27,28,37,39–44]. Most circular features are distorted by the oblique lighting [42]. ii) Vertical and ring light illumination requires additional experience from the firearms examiner [37]. The advantage of the ring light is that it gives a good picture of the marks in the firing pin impression, which are hardly illuminated (or overexposed) when a sidelight source is used [23]. Contrary, images of the breech face marks are much more distinctive when viewed with sidelight [23]. iii) More modern instruments use a form of lighting called ‘Ultrapak’ or co-axial lighting system. This type of system requires a shadowless but incident lighting system [15]. Clear and flare-free imaging of fine traces on very shiny metallic or plastic surfaces can be achieved by using a co-axial illumination unit like the one fitting the new comparison microscope Leica DMC [15,45,46].

B. Confocal microscopy

The image formed in a confocal reflection microscope displays the true 3D structure of the investigated object. Figure 2a shows, in gallery form, the raw confocal data constituting the 3D image of a land impression on bullet B1. This figure shows 20 single optical sections, acquired with a separation of 5.25 microns. The images are colour-coded: black, red and yellow respectively mean a low, intermediate and high reflection intensity. For example the red regions in the image labelled ‘36.8 μm’ correspond to those regions of the bullet which are in focus at that specific bullet-to-lens separation. Inspection of the other optical sections show the different regions of the bullet which come in and go out of focus at different object-to-lens separations. Figure 2b



FIGURE 7 Rotated surface rendering of a 3D confocal image of the top of the firing pin impression of cartridge case C2, showing the cartridge case surface (white regions) and the curvature of the impression. Objective lens: 10x, image dimensions: 0.9x0.9x0.5 mm.



FIGURE 8 Extended focus projection of a 3D confocal image of the breech face mark of cartridge case C2. The individual striae are clearly visible. Objective lens: 40 x (oil immersion), image dimensions: 230x230x32 micron.

shows an extended focus projection of a 3D image of the same land impression shown in Figure 2a. The image in Figure 2b was acquired with a lower magnification lens, so that the entire land impression can be seen in a single image. Three tests were performed to show that the images taken with a confocal microscope are, in first order, independent of the relative angle between the bullet and the microscope objective lens. i) After the acquisition of the image in Figures 2a and 2b, we removed the bullet and repositioned it in such a way that its orientation was approximately, but not exactly, the same as before its removal. The extended focus projection of the image acquired after repositioning is shown in Figure 2c. Comparison of Figures 2b and 2c shows that despite the fact that the orientation of the bullet with respect to the objective lens was not exactly the same, the same striation marks can be seen in both images. Minor intensity differences between the extended focus images taken before and after the repositioning are caused by a different relative height of the individual optical sections on the bullet in both situations. However, the important point we would like to make is that the same features can be seen before and after repositioning.

ii) Figure 2d shows the extended focus projection of an image of the bullet after removal and deliberate gross misalignment with respect to the position used in Figures 2b and 2c. Comparison of Figure 2d with 2b and 2c shows clearly different structures. This is caused by new features which are not present in the fields of view of Figures 2b and 2c as well as shadowing effects which mask certain features in Figures 2b and 2c.

iii) The object orientation independence of confocal images is also demonstrated by the visualisation of both horizontal and vertical striation marks without changing the settings of

the microscope. Figure 3a shows an extended focus projection of a 3D confocal image of the breech face mark of cartridge case C1. Clearly visible are the fine lines, both in the vertical and the horizontal directions. After acquiring this image, the cartridge case was removed, rotated 90 degrees clockwise and imaged again, using the same settings of the microscope. An extended focus projection of the 3D data was calculated and rotated 90 degrees counter clockwise. The result is shown in Figure 3b. Comparison of the images shows that for both orientations of the cartridge case, the same horizontal and vertical features as well as the fine marks can be seen. This is in contrast with pictures made with the conventional microscope, as illustrated in Figures 3c and 3d.

Figures 2 and 3 illustrate that the features present in a confocal image are in first order independent of the orientation of the object. Stated differently, if two objects are to be compared, the orientation of the two objects is in first order not important. Only if the objects are grossly misaligned, the interpretation of correspondences and differences between the two images will become difficult.

2. The intensity of the light beam

A. Conventional microscopy

An important factor in the study of striation details is proper type of illumination. In fact, for specimen comparison it is imperative that the lights strike the bullets or cartridge cases not only at the same angle but also with alike intensities [22,29]. A variable light source intensity is used to optimise any image variation that would be seen with oblique lightning [11,27,44]. Usually, low level lighting reveals best the variations of the marks [9]. To overcome the disadvantage of the light-dependency of the appearance of marks, standardised lighting conditions and specific illum-

nation protocols were proposed mostly for database purposes [10,20,24,47]. Baldur [48] describes a method and apparatus which monitors the position of a bullet under optical observation for forensic purposes and adjusts its position as required.

B. Confocal microscopy

In confocal microscopy the image is independent of the illumination intensity of the laser. When using a high laser intensity, the intensity of the reflected light will be high. In order to prevent that this high reflection intensity will saturate the confocal photodetector, a low detector gain should be chosen. Correspondingly, a low laser intensity should be combined with a high detector gain, to obtain a significant detector signal, with a low noise content, from the low reflection intensity.

In general, it is advisable to use a high laser intensity and a low detector gain, since high detector gains increase the noise content of the confocal image.

The actual combination of laser intensity and detector gain is not important for the visibility of features in a confocal image, provided that the image is neither saturated nor dominated by noise contributions. This aspect of confocal microscopy is demonstrated in Figure 4, which shows two extended focus projections of the breech face mark of cartridge case C1. Images 4a and 4b were acquired with a low and a high laser intensity, respectively. Comparison of the images confirms that the same features are present in both images. The small intensity differences between the images have been discussed earlier (see section 1B).

3. Type of light used

A. Conventional microscopy

Currently, different types of light sources are available on the market. Depending on the temperature of the light used, the surface is more or less strongly illuminated and, therefore, certain details can appear or disappear [22,49,50].

Three types of high-intensity lamps are usually fitting conventional microscopes, which may be compared on the basis of spectral output, cost, ease of operation and intensity [51].

1. Tungsten-halogen lamps produce a smooth, continuous spectrum in the visible range.
2. Mercury-arc lamps emit a high intensity line spectrum in the near UV, violet and green, with more moderate levels in the blue.
3. Xenon-arc lamps provide a continuous spectrum in the near UV and visible.

'Cold-light' illumination by fibre optics is also available, enabling one to separate the source from the specimen [15,46,50–52].

In using the comparison microscope, it is sometimes best to have the marks illuminated with a soft light set [37]. More often, however, stronger light from micro-illuminators is used [2,37]. Fluorescent or diffused tungsten light sources (3000 K) are less satisfactory [2,53].

B. Confocal microscopy

In confocal microscopy generally a laser is used as the illumination source. Nowadays, the available laser colours vary from ultraviolet (250 nm), via visible (from 400 – 800 nm) to far infrared (1000 nm). To obtain optimum image quality, a single laser line (as opposed to a combination of laser lines) should be used for image acquisition. The relation between laser colour and image quality (or visibility of the marks) will be discussed in more detail in section 7.

4. Colour and reflectivity of the marks

A. Conventional microscopy

The material (nickel, copper, lead, etc.) on which the traces are present has a great influence on the comparison [22]. For example, Winchester has produced a bullet with an innovative satin black finish (Black Talon™) on which the difference between the shadows of the traces and the support itself is difficult to establish [33,50]. It follows that if surfaces to be compared have different reflecting properties, traces which are the same may appear quite different [4]. This often happens when no firearm is available for production of comparison firings. In those cases, the forensic scientist has to compare evidence markings from different crime scenes which can be present on different kinds of supports [4].

Moreover, different areas on the marks will frequently differ widely in contrast and colour [2–4,13,37]. They can be i) dulled by a film of oxidation or corrosion (due to the influence of the climate and also to the handling by firearms experts) [2,4,37,54], ii) influenced by terminal (human) ballistics, resulting in an unevenly stained or oxidised appearance [2,55] or iii) covered with other very thin deposits, such as hot propellant gases [2,11]. These marks will not reflect the same amount of light and this phenomenon can be overcome by using several techniques such as unrolling, coating, casting or replicating [2,4,33,37,54,56]. Specular reflection produced by the shiny metal can also be eliminated by using the same techniques as well as by using polarisation filters [2,14,31,37,46,50,53,57,58].

Unrolling, coating, casting and replicating techniques are also used in order to observe traces on dark supports (as for example, the Winchester's Black Talon™ bullets) or on transparent or translucent supports [43,50,55].

B. Confocal microscopy

The effect of colour and reflectivity of the support on confocal images is illustrated in Figures 5a and 5b, which show two extended focus projections of casts of the breech face marks of cartridge case C1. Two casting materials with different colours (blue and green) but similar (as determined by visual inspection) reflective properties were used. From the confocal images, it is evident that despite the different colours, the same surface features can be seen. Figure 5c shows an extended focus projection of the same marks using a grey casting material which displayed more diffuse reflection (again determined by visual inspection) than the casting materials used in Figures 5a and 5b. The same surface features can be seen but the signal-to-noise ratio is much lower in Figure 5c due to the higher diffuse reflection

of this particular casting material when compared with those in Figures 5a and 5b.

Figures 5a–5c demonstrate that the visible features in a confocal image are only determined by the height structure of the object and not by the colour of the support. The visibility of the features is related to the reflective properties of the support.

5. Characteristics of the marks

A. Conventional microscopy

Sometimes, bullets or cartridge cases may be seriously damaged or their characteristic topography is too shallow for conventional optical examination [4, 8, 59]. For example, the striations produced by Glock barrels, which lack conventional land and groove rifling, are frequently too scant and peculiar to be compared using a reflected light microscope [50, 60]. At the other extreme, bullets and cartridge cases can have highly three-dimensional structures, which are nearly impossible to investigate by conventional light-optical methods [4, 7, 8, 50, 59]. As an example, firing pin marks are often too deep for optical examination; in fact, only a small portion of the impression is in focus at the same time [61].

B. Confocal microscopy

Certain features which are difficult to visualise in conventional microscopy can be efficiently visualised with confocal microscopy.

Figure 6a shows single sections, acquired at different heights, of the bottom of the firing pin impression of cartridge case C2. The figure confirms that the firing pin impression is elliptically shaped and the increase of the size of the ellipses, together with the separation of the confocal sections, gives an indication of the curvature of this segment of the firing pin impression. Figure 6b shows an optical cross section, clearly displaying the impression's curvature. The full 3D shape of the bottom of the firing pin impression is shown in Figure 6c, using the surface rendering data visualisation method. The image is (mathematically) tilted with respect to the direction of view, so that the complete spatial form can be appreciated better.

The firing pin impression shown in Figure 6 was too deep to be completely visualised in a single 3D image. After visualising the bottom, a 3D image of the top of the firing pin impression was acquired. The full 3D shape of the imaged segment, tilted for viewing convenience, is shown in Figure 7. By combining the images in Figures 6 and 7, complete and detailed insights in the full spatial form of the firing pin impression can be obtained.

In Figure 8a we show an extended focus projection of a 3D image of striations in the breech face mark of cartridge case C2. From this projection, the individual striations are clearly visible. It should be noted, however, that only the 2D shape of the striations could be visualised and no information on the depth structure of individual marks could be gathered. This indicates that the height of individual striations is below the standard confocal microscopic axial resolution which with the objective lens and laser wave-

length used (see also section 7) is approximately 700–800 nm.

The smallest features that can be detected in confocal microscopy are of the order of the dimensions of the illumination spot. Features smaller than the spot dimensions are 'smeared' by the illumination spot and cannot be resolved. When one knows the exact resolution function in x, y and z co-ordinates, it is possible to correct for this smearing by applying a deconvolution; this technique might reveal the 3D shape of the individual striations [62].

The results in Figures 6–8 indicate that both shallow and deep marks can be visualised well with confocal microscopy. For a complete and detailed overview of large traces it might be necessary to combine several 3D confocal images. One way to implement this image combination is the so-called confocal 'stitching mode', in which first, one 3D image is acquired, then the microscope stage is moved to an adjacent region, a second image acquired, etc. It should be pointed out that the implementation of this stitching mode requires stringent stage mechanics and should be combined with stringent 3D alignment procedures to acquire the images. If this is not available, overview images of large structures are best acquired with conventional microscopy, with all its disadvantages (see also section 7 and the 'discussion' section).

Another interesting evolution in this respect is the so-called 'confocal microscope/macroscope'. This apparatus is capable of 3D imaging large specimens (up to 7.5 x 7.5 cm) with moderate lateral and axial resolution (macroscope mode) and then zoom into 3D to examine small areas of interest at high resolution (microscope mode) [63, 64].

6. Form of the support of the marks

A. Conventional microscopy

The comparison process is by no means easy, as the observation of cylindrical surfaces under even low magnification requires special methods of illumination and extremely precise techniques [65]. Many of the marks to look at have curved surfaces or are on the curved surface of bullets or cartridge cases, e.g. a firing pin impression, or an extractor mark situated inside the rim of a cartridge case [66]. Curved surfaces are particularly troublesome since they will reflect a glare in certain areas regardless of the angle of illumination [53]. Various casting methods allow to reduce or obviate the difficulties inherent in the direct observation of curved or inaccessible surfaces by reflected illumination [31, 67].

B. Confocal microscopy

With confocal microscopy, only the parts of the bullet which are in focus are measured. Curved and/or deformed surfaces present no fundamental limitations for confocal microscopy, as was clearly demonstrated in the preceding sections.

7. Compromises among resolution, magnification, depth of field and sampling area

A. Conventional microscopy

Because microscopes form enlarged images, it might

appear that magnification is the principal aim of microscopy. Beyond a certain level of enlargement, however, no further detail can be detected: the useful magnification is exceeded. Useful magnification increases cannot be obtained without attention to the requirements of resolution [5] which is the detail actually revealed in the image of a given object. In optical microscopy, the resolution of the microscope is determined by the objective lens used and the wavelength of the illuminating light [51]. The resolution of a conventional optical microscope proves to be about 500 nm, the magnifying power ranges from 6x to 1000x and the depth of field is 2–3 mm for high numerical aperture, high magnification lenses [68].

There is always a price to pay when moving to a higher magnification: the field of view becomes limited and the depth of field is reduced [5,59,66,68].

According to several authors, light microscopy does not possess a sufficient range of magnifications, enough resolution and depth of field for the visualisation of marks on bullets and cartridge cases. This is particularly true when curved surfaces are under study [2,8,32,59,60,68–70].

A diaphragm is absolutely necessary for good results in conventional comparison microscopy, because when it is closed down to a small opening the depth of field is increased and the traces which are slightly above or below the point of focus can be clearly seen [70]. However, by using a too small opening of the diaphragm, diffraction patterns can appear.

B. Confocal microscopy

In confocal microscopy, one generally speaks of two resolutions: the lateral and the axial resolution. The lateral resolution is defined as [17]:

$$r_{lat} = \frac{0.6\lambda}{NA} \quad (1)$$

in which λ is the wavelength of the illumination light and NA is the numerical aperture of the lens. The lateral resolution can be interpreted as the minimum separation between two objects for them to be perceived as different objects. For a 100x, NA=1.3 lens and 500 nm illumination light, the lateral resolution is 230 nm (conventional microscopy: 500 nm). The axial resolution is defined as [17]:

$$r_{ax} = \frac{1.505n\lambda}{NA^2} \quad (2)$$

in which n is the refractive index of the medium between objective lens and object (air for air lenses and immersion oil for oil lenses). The axial resolution is the confocal analogue of the conventional depth of field: it describes the minimum detectable height difference between two objects. For a 100x, NA=1.3 lens, 500 nm illumination light and standard immersion oil, the axial resolution is 675 nm (conventional microscopy: 2000–3000 nm). Equations (1) and (2) show that for optimum resolution, the wavelength of the

illumination light should be as short as possible and the NA of the objective lens as high as possible.

In practice, confocal microscopes are equipped with lasers emitting in the visible range (400–650 nm) and the difference in resolution when using different laser lines is not significant. The objective lens used is, through both magnification and NA, of course an important factor determining the visibility of structures characterising an object.

As in conventional microscopy, in confocal microscopy a higher magnification corresponds to a smaller field of view and a better axial resolution (i.e., a smaller depth of field) due to the fact that higher magnification lenses generally have a larger NA. It should be pointed out that the main difference between conventional and confocal microscopy is that whereas the reduction of the depth of field with high NA lenses is a disadvantage in conventional microscopy, it is an advantage in confocal microscopy: the higher the NA of the lens, the better the depth resolution and the smaller the smallest detectable height difference [69,71].

For both conventional and confocal microscopy, the ‘stitching mode’ (see also section 5) is a solution to the compromise between magnification, resolution and depth of field on one hand and sampling area on the other hand.

8. Lens aberrations

A. Conventional microscopy

Ideally, lenses redirect radiation in such a way that all the rays that diverge from a given object point are reunited at a corresponding image point [51]. The departures in performance between actual and hypothetical ‘ideal’ simple lenses are commonly referred to as ‘aberrations’. These aberrations do not arise from defects introduced during the manufacturing process. They are inherent even in a perfectly made simple lens [5]. Real lenses are commonly made with surfaces which are sections of spheres, because such surfaces can be ground with great accuracy. However, even a perfectly made simple lens of this type will not bring all rays emanating from an object point to a common focus. There are five important aberrations.

1. Spherical aberration is related to the fact that zones of the lens which are closer to its periphery have shorter focal lengths than zones near the axis of the lens. Spherical aberration is defined in terms of the difference in focal lengths between the axial and marginal zones of the lens [5,51,52,68].
2. Coma leads to images with comet-like figures, consisting of a bright head and a diffuse tail [51].
3. In astigmatism, the focal length varies as a function of the angle at which rays are incident. Both coma and astigmatism affect the imaging of off-axis object points, such that the deterioration of the image increases as the effective aperture of the optical system increases [51,52,68].
4. Curvature of field is seen when a lens images a planar object as a curved surface [5,51,52].
5. Distortion is an aberration in which the magnification of peripheral zones of the image is larger or smaller than at the axis. This aberration poses a critical problem in the obser-

vation of three-dimensional surfaces [51–53,72]. Distortion is further subclassified as barrel and pincushion distortion. Barrel distortion occurs when the magnification falls off when moving from the centre of the field toward the periphery. In cases where the magnification increases toward the edge of the field, the term pincushion distortion is used [5, 52]. Distortions can follow from the quality of the objectives as well as from mistakes during the focusing of the surface. It should be highlighted that, especially when observing non-flat surfaces, it is essential that both surfaces being compared are exactly in the same position with respect to the objectives [14].

Besides these so-called ‘monochromatic aberrations’ (also referred to as ‘geometric’ or ‘third-order’ errors), which exist for any specified colour and refractive index, additional image defects occur when the light contains various colours [51,52]. These are:

1. longitudinal chromatic aberration, which describes the variation of focal length with wavelength. Since refractive index normally increases as the wavelength decreases, a lens exhibiting uncorrected longitudinal chromatic aberration focuses shorter wavelengths closer to itself [5,52,68].
2. lateral chromatic aberration, which is defined as the variation of magnification with wavelength [5,52].

Simultaneous corrections for geometric and chromatic aberrations appear to be impossible [51]. Spherical aberration and coma can be corrected by using a contact doublet of the proper shape; astigmatism and curvature of field require for their correction the use of several separated components and distortion can be minimised by the proper placement of a stop [52].

B. Confocal microscopy

The aberrations which do not depend on the colour of the illumination (the monochromatic or geometric aberrations) are, in principle, also present in confocal microscopy. As confocal microscopy in reflection mode exploits laser light having one wavelength, chromatic aberrations are absent.

9. The influence of the expert

A. Conventional microscopy

As the best comparison techniques are determined by experimentation with the particular marks being compared, the results depend strongly on human aspects like accuracy, personal preferences, skill and persistence of the examiner in choosing and adjusting the different viewing conditions: there are a lot of parameters to optimise and a skilled expert is required to effectively set up the microscope [1,4,9,21,24, 26,33,37,42,50]. The amount of effort necessary to compare specimens on the comparison microscope limits the use of ballistic evidence to a confirmatory role, rather than an investigative role [42].

From a time-management point of view, setting up the microscope can be very time consuming, whereas recording of the results (with a photo camera or CCD camera) is fast.

B. Confocal microscopy

In confocal microscopy, no special set up procedures are required: one places the object to be imaged under the

objective lens, optimises the microscope and acquires the image. Optimisation entails:

1. Selecting a combination of laser intensity and detector gain in such a way that no detector saturation or signal underflow occurs.
2. Selecting the dimension of the image (ranging from 128 x 128 up to 2048 x 2048 pixels in state of the art microscopes). For each objective lens there is an optimum image size. In practice, a 512 x 512 pixel image is used.
3. Selecting the number of confocal sections. For an optimal 3D representation the number of sections is given by the ratio of the depth of the feature to be measured and the optimal section spacing which is half the axial resolution of the given objective lens [17].
4. Selecting the scan speed. The slower the scan speed, the less noise the image will contain. Modern microscopes are equipped with variable scan speeds: fast scan speeds are used to rapidly inspect the sample and slow speeds to accurately image the selected object segments.

Depending on the choices for points 2–4, the actual scanning of the full 3D confocal image can take from less than one minute up to five or ten minutes. This means that the situation is reversed when compared with conventional microscopy: setting up the microscope is fast, whereas image acquisition can take up much time.

10. The establishment of photographs

A. Conventional microscopy

The eye is capable of a good deal of accommodation but when an attempt is made to photograph certain features, the difficulty of recording becomes manifest [7]. In fact, it is impossible to reproduce perfectly in a photograph, which is a two-dimensional surface, the illusion of a three-dimensional space: the camera represents a single eye and can do no better than to give a monocular view of a subject. In the absence of binocular vision there is no depth except in memory [33,53].

Many firearms experts are unwilling to use comparison microscope photographs when illustrating reports or testifying in court because the out-of-focus areas are hard to explain to jurors, and defence attorneys frequently harp on this deficiency in an effort to discredit the witness [15,73]. Moreover, the data obtained in this way are difficult to exchange, even within the same laboratory.

B. Confocal microscopy

In confocal microscopy, the stack of confocal sections is used to generate, on a two dimensional surface such as a laser print or a photograph, a three dimensional representation of the imaged mark (see Figure 7). The 3D structure of the mark is directly evident from the 3D representation, without relying on the binocular capabilities of the human eye.

In confocal microscopy, the data which are observed and measured are the same as those presented in court. In addition, the confocal data is inherently digital and the images can be presented in standard file formats so that exchange of the data, both within the laboratory and outside, is straightforward.

11. Qualitative/quantitative measurements

A. Conventional microscopy

The use of conventional microscopy can only lead to qualitative information about the presence or absence of characteristic features, such as striation marks. The use of shadowing to extract features is dependent on subjective operator interpretation and this represents a major drawback to what is called 'contour analysis' [9, 20, 24]. In fact, the concept of contour analysis is crucial to tool mark comparisons because a series of striations possesses more information than the 'lines' which may be matched utilising a comparison microscope [9]. The individual striations should exhibit similarities in contour (height, width) if they were formed by the same tool and an examination procedure which yields this additional information could also detect the pressure differences affecting the depth of the marks [9]. Regardless of how well illuminated a specimen may be, it is generally impossible to accurately evaluate its contour through conventional microscopic evaluation [4].

B. Confocal microscopy

Confocal microscopy permits not only a direct examination of a surface but also generates data that can provide mathematical descriptors [6, 20, 26]. In fact, in the raw confocal image, the differences in intensities are directly related to differences in object height. Since the images are intrinsically calibrated in the x, y, and z directions (both the (projected) size of a single image pixel and the separation between two optical sections are known), roughness parameters can be extracted from the confocal data set and statistically processed for class or individual characterisation [6, 20]. In addition to all common 2D and 3D surface roughness parameters, Fourier transforms and correlation may be performed. This possibility of gathering quantitative spatial information of structures with dimensions in the micron range implies that confocal images are well suited as the basis for mathematical analyses, such as contour analysis.

12. Environmental light (subdued lighting)

A. Conventional microscopy

Few authors discuss this parameter. According to the experiments carried out with Drugfire™, the influence of the environmental light during the capture of images seems little [24].

B. Confocal microscopy

In confocal microscopy, no significant effect of environmental light is to be expected when it enters the microscope and is detected; in fact, the intensity of the detected environmental light will be insignificant compared to the reflected laser light. However, for good practice, the lights should be turned off while measuring a sample.

Special complications related to confocal microscopy

1. The use of oil immersion lenses

Certain objective lenses are specially designed for oil immersion. Correct use of such lenses requires the use of a cover glass (having a correct refractive index and thickness) and immersion oil between objective lens and cover glass

and between cover glass and object to be observed. If an object is immersed in a medium with a refractive index other than that of the specified immersion oil, the apparent axial dimensions of the observed object can differ significantly (a factor 2.5 for water as the immersion medium) from its actual axial size. This artefact is called 'focal anomaly' and is caused by the fact that, under incorrect examination conditions, the focal plane does not follow the axial translation of the microscope table, but, generally, moves over a smaller distance. The precise value of this anomaly depends on the index of refraction of the immersion medium and on the distance between the object and the interface. Since especially the latter parameter is not always known, this means that for efficient quantitative object size determination using confocal microscopy, correct immersion conditions should be employed [74].

2. Diffraction effects

The illumination pattern in a confocal microscope is not a single spot but consists of a central spot, surrounded by concentric illumination shells. The surrounding shells are a direct consequence of optical diffraction effects at the apertures of the objective lens and cannot be eliminated. The consequence of the presence of these shells (or, more accurately, the dark concentric shells between the central spot and the illumination shells) is that when a planar object is 3D imaged in the confocal reflection mode, at certain axial positions of the object, dark segments can arise in the acquired confocal section.

For most applications these dark segments will not be important. For example, if the height profile of a certain mark is of interest, one can fill in the missing data using information from the upper and lower confocal sections. An alternative to interpolation is image reconstruction (as described in section 5) at the 'cost' of extra confocal measurements and computer time to carry out the correction [62].

3. Real time observation

In order to obtain real-time three-dimensional imaging in confocal microscopy, Wilson [75] describes two different approaches. The first approach uses the traditional confocal optical system and exploits aperture correlation techniques which enable many closely packed confocal systems to image in parallel with no cross-talk between the adjacent confocal channels. The second approach is based on the capability of a conventional microscope to retrieve a thin optical section; this can be achieved by projecting a single spatial frequency fringe pattern onto the object to be visualised. This system has also the practical advantage to be able to produce the conventional image at the same time as the optically sectioned one [75].

4. Direct comparison of the specimens

The confocal microscope has the disadvantage that the two specimens under observation cannot be compared at the same time. Instead, each specimen must be mounted and observed individually.

Discussion

In this paper a comparison is made between the capabilities

of conventional and confocal microscopy for visualising traces on bullets and cartridge cases. It was demonstrated that, in addition to providing true three-dimensional information on the microstructure of the investigated marks, confocal microscopy provides solutions for five main limitations of conventional microscopy.

First, the features visible in a confocal image are independent of the relative orientation of the object and the light source. This means that if the object contains striation marks which are not parallel to each other (or even the extreme case of striations at right angles), all striations can be visualised in a single 3D confocal image, with equal visibility. In addition, the positioning of the object is in first order not critical: one can acquire a 3D confocal image and if, in a later stage of the investigation for whatever reason, another image is required, the same features visible in the first image can be imaged, provided that the object is not grossly mispositioned.

Second, in standard confocal microscopy (with a limited number of different visible laser wavelengths), acquired images are effectively independent of the illumination wavelength. The specific choice of the illumination intensity is of no importance as long as no detector over-or underflows occur.

Third, the colour of the support does not have any influence on the features visible in the confocal image. The reflectivity, however, influences the signal-to-noise ratio of a confocal image: the more diffusely reflecting the lower the signal-to-noise ratio will be.

Fourth, both shallow marks (height less than 2–3 micron) and deep marks (depth more than 50 micron) present difficulties for imaging in conventional microscopy. In confocal microscopy the height range of objects is significantly enlarged. In standard confocal microscopy objects with axial dimensions ranging from approximately 700 nm to about 120 micron can be visualised effectively with high magnification, high NA lenses typically suited for bullet and cartridge case examination. For large marks, it might be necessary to use the ‘stitching mode’. In addition, the form of the support (either planar or curved) is not important for the visibility of the marks.

Fifth, whereas in conventional microscopy it is difficult (if not impossible) to represent the 3D structure of a mark on a photograph, in confocal microscopy any representation of the confocal data (either in the form of a simple gallery or a more sophisticated surface rendering) directly displays the full three-dimensional microstructure of a mark on a two-dimensional photograph.

General attractive features of confocal microscopy are i) its multipurpose applicability, ii) the availability of versatile 3D image processing software, iii) the combination of a high quality optical microscope—allowing to see a direct image—with an indirect image building device and iv) the relatively short image acquisition times when compared to set-up times for conventional comparison microscopy. These specific advantages over conventional microscopy, come with some specific complications of confocal microscopy.

First, for high resolution overview images (i.e. images comprising a larger field of view than accessible with a single confocal section) the so-called ‘stitching mode’ should be employed, which combines several 3D images to a larger composite image. For conventional microscopy it is relatively straightforward to implement such a ‘stitching mode’ because only 2D stage translations and mathematical image alignment procedures are required. In the confocal case, the 3D stage position should be taken into account and after data acquisition, the images must be aligned in three dimensions.

Second, currently confocal microscopes are relatively expensive and, although the microscope is relatively straightforward to operate, a well-trained technician with more than average know-how of the details of confocal image formation should be available to maintain and calibrate the microscope and to image structures which are difficult to visualise with standard confocal set-ups.

Third, up to date no real time observation station is commercially available for microscopic comparisons functioning on the principle of confocal microscopy.

From a forensic point of view perhaps the most interesting property of confocal microscopy is that it gives access to quantitative three-dimensional information of the imaged object. This means that, with the confocal data as the basis, it is possible to extract quantitative structural parameters characterising the investigated surface, such as feature dimensions (height and width of marks), surface curvature and generalised surface roughness parameters. Comparison of different marks could then be based on the quantitatively determined surface parameters, rather than on the shadows cast by the surface features. Stated differently, the availability of confocal data from which quantitative surface parameters can be determined i) eliminates subjectivity and ii) makes it easier to automate the comparison process, for example by building databases based on the surface parameters in combination with intelligent search algorithms. In fact, the major breakthroughs in forensic firearm examination will probably come from three-dimensional topography visualisation techniques (aided with cheap computing power and the growing field of nanotechnology) along with more flexible software. The challenge is not only to technologically develop a solution, but to do it in a way that will enable most of the forensic laboratories to acquire and operate such systems [76]. The experiments described in this paper indicate that confocal microscopy is an interesting 3D visualisation technique with both promising and challenging prospects for application in forensic firearm examination.

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