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## Flattening mountains: micro-fabrication and bullet lateral striae analysis.

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### Abstract

The application of replica molding has proven to be a valuable tool in the analysis of different forensic evidences in particular for its ability to extract the toolmarks from complex sample surfaces. A well known problem in the analysis of ballistic evidences is the accurate characterization of the lateral striae of real bullets seized on crime scenes after shots, due primarily to impact deformations and to unpredictable issues related to laboratory illumination setup. To overcome these problems a possible way is to confine over a flat surface all the features still preserving their three dimensionality. This can be achieved by a novel application of replica molding performed onto the relevant lateral portion of the bullet surface.

A quasi-two-dimensional negative copy of the original tridimensional indented surface has been thus fabricated. It combines the real tridimensional topography of class characteristics (land and groove impressions) and of individual characteristics (striae) impressed by rifled barrels on projectiles, moreover with the possibility of quantitative characterization of these features in a planar configuration, that will allow one-shot comparison of the "whole striae landscape" without the typical artifacts arising from the bullet shape and the illumination issue.

A detailed analysis has been carried on at the morphological level by standard optical and scanning electron microscopy, while the 3D topography has been characterized by white light optical profilometry.

A quantitative characterization of toolmarks of bullets derived from ammunitions shot by guns of large diffusion, as the Beretta 98 FS cal. 9 x 21 mm, has been performed and will be presented ranging between the whole landscape and the sub- $\mu\text{m}$  resolution. To investigate the real potentiality of this technique, the experiment has been extended to highly impact-deformed projectiles.

### Introduction

Ballistic samples are among the most crucial evidences that are nowadays used for solving criminal cases. In this frame a key role is played by the search for characteristic features on ballistic evidences

found on the crime scene. This kind of toolmarks is at the base of all the comparisons performed for identifying and associating the evidences to a specific weapon [1]. Different marks are produced by a firearm upon shooting: the firing pin, the breech face and the ejector hit the case head leaving on its surface indentation marks, while the gun rifled barrel, to impart the well known gyroscopic motion, leaves on bullets class characteristics, constituted by a sequence of lands and grooves, and groups of microscopic striations univoquely related to a specific firearm [2].

A great effort has been devoted by forensic scientists to understand [3, 4] and to develop increasingly accurate methods for analysing these features [5] [6]. In particular the striae pattern has been deeply studied [7][8], because fired projectiles are the evidences more often found on crime scenes where have been used firearms. The most typical forensic analysis of bullets is based on the comparison of the striae belonging to different evidences to assess if they can be associated to the same firearm. This process is usually performed by forensic in real time comparative microscopes that enable alignment of the correspondent features present on two samples. Significant improvements have been made on these instruments to reduce any possible artifacts intrinsic to the white light reflection optical imaging. Few issues are still present, as it has been underlined in seminal work ([9] [10] [11]), and are mainly connected to the illumination and to the shape, generally not planar of the sample. When dealing with a series of quasi-parallel grooves with different depth, a non proper illumination (a too grazing incidence) can enhance some details while hindering others, thus distorting or even changing the detected striae pattern. To overcome these problems it has been recently proposed by some research groups a shift towards a real three-dimensional imaging, because in such manner the topography of a toolmark is not influenced by the above-mentioned factors [12] [7, 13]. Following the same approach, even automated ballistic identification systems (e.g. IBIS) are presently mapping the 3D topography of case cartridge and bullets.

A second important issue in comparing bullet striae with traditional techniques is due to their intrinsic nature of being a surface distribution of marks over a macroscopically curved object. The projectile has in fact a cylindrical shape that is grooved by a rifled barrel only on the lateral portion, thus on the one hand a non perfect alignment of its axis with the rotation axis of the microscope manipulator may induce distortions of the striae appreciation, as it has been well described by Bonfanti and coworkers [9]; on the other hand in a common microscope the striae do not lay in the same focal plane and, without automated Z-stacking photography systems, it is not easy to acquire well focused images. All these drawbacks are even more crucial when dealing with real evidences that upon impact assume a strongly deformed shape.

In this work we propose a method to overcome the above illustrated issues base on an refined version of a protocol presented elsewhere [14] where high-resolution imaging was combined with extremely accurate replicas [15, 16] of fired case head to identify and associate the firing pin marks down to the nanometer level. Here a similar procedure is exploited to produce thin planar replicas of the whole lateral landscape of a bullet that displays, in a straight plane, the three dimensional topography of all the striae sequence. We think that this “replica approach” can be a strong help in the bullet analysis, because it preserve the three-dimensional nature of the grooved striae, thus allowing accurate topographical exams, removing, at the same time, the problems related to long scale curvatures of evidencens or to acquisition, by classical optical microscope, of correct images of highly deformed projectiles. (e.g. “mushroomed” bullet).

## **Materials and methods.**

### *Ammunition, Shooting tests, and Sampling*

Fired bullets were kindly provided by the forensic Regional unit of Italian State Police (Gabinetto di Polizia Scientifica per l'Emilia Romagna, Bologna); the gun cartridges cal. 9x21 mm and cal. 7,65 Browning (Fiocchi, Lecco, Italy and Winchester, New Haven, USA) have been fired by two different pistols Beretta, model 98FS (Beretta, Gardone, Italy) and a submachine gun CZ, model Scorpion VZ61.

### *Replica Molding*

Polydimethylsiloxane (PDMS) and its curing agent (Sylgard 184, Dow Corning, Midland, USA) were mixed in ratio 10:1, degassed to eliminate air bubbles and poured on the bullet with a iron tongue. The movement of the casting must be circular to allow evenly cover the surface avoiding air bubbles. Curing was performed at 120°C for 30 minute, in a standard thermostatic oven. After peeling off, the PDMS replica was rinsed in ethanol, sonicated for few minute, and gently dried and spread on the bearing under nitrogen flux . It allows to get a replica clean and well bonded to the substrate.

### *Optical microscopy*

Optical images were collected by with a Nikon microscope (Nikon, Tokyo, Japan) operated in bright field. The reconstruction of the full bullet lateral striae landscape has been performed using the large image grabbing tool of the software NIS-Elements-D-3.2-64-bit (Nikon).

Optical comparisons were realized by a forensic microscope Leica FSC (Leica Microsystems, Heerbrugg, Switzerland), equipped with a Leica DFC 500 12 Megapixel digital camera; samples' images were collected in TIF format by Leica image software LAS ver. 3.8 and finally optimized by Adobe Photoshop ver CS3.

### *White light optical profilometry*

Optical profilometry has been performed with a SmartWLI profilometer (GBS GmbH, Ilmenau) equipped both with a 10X and 20X objective. Topographic images of the samples have been collected over areas previously selected on the basis of the optical microscopy comparison. The image acquisition and analysis (extraction and averaging of 50 neighbouring profiles) has been performed using the software MountainsMap Premium by Digital Surf (Besancon, France).

### *Scanning Electron Microscopy (SEM)*

SEM analysis were performed using both a SEM-FEG Hitachi S4000 ( $V_{\text{ext}}= 20 \text{ KeV}$ ;  $i=\mu\text{A}$ ) (Hitachi, Tokyo, Japan) and a SEM Phillips 515 ( $V_{\text{ext}}= 20\text{-}28 \text{ KeV}$ ;  $i=\mu\text{A}$ ) (Philips, Amsterdam, The Netherlands); before imaging, samples were sputtered with a thin conductive gold layer (20 nm) in order to eliminate the electrostatic effects.

### *Image analysis:*

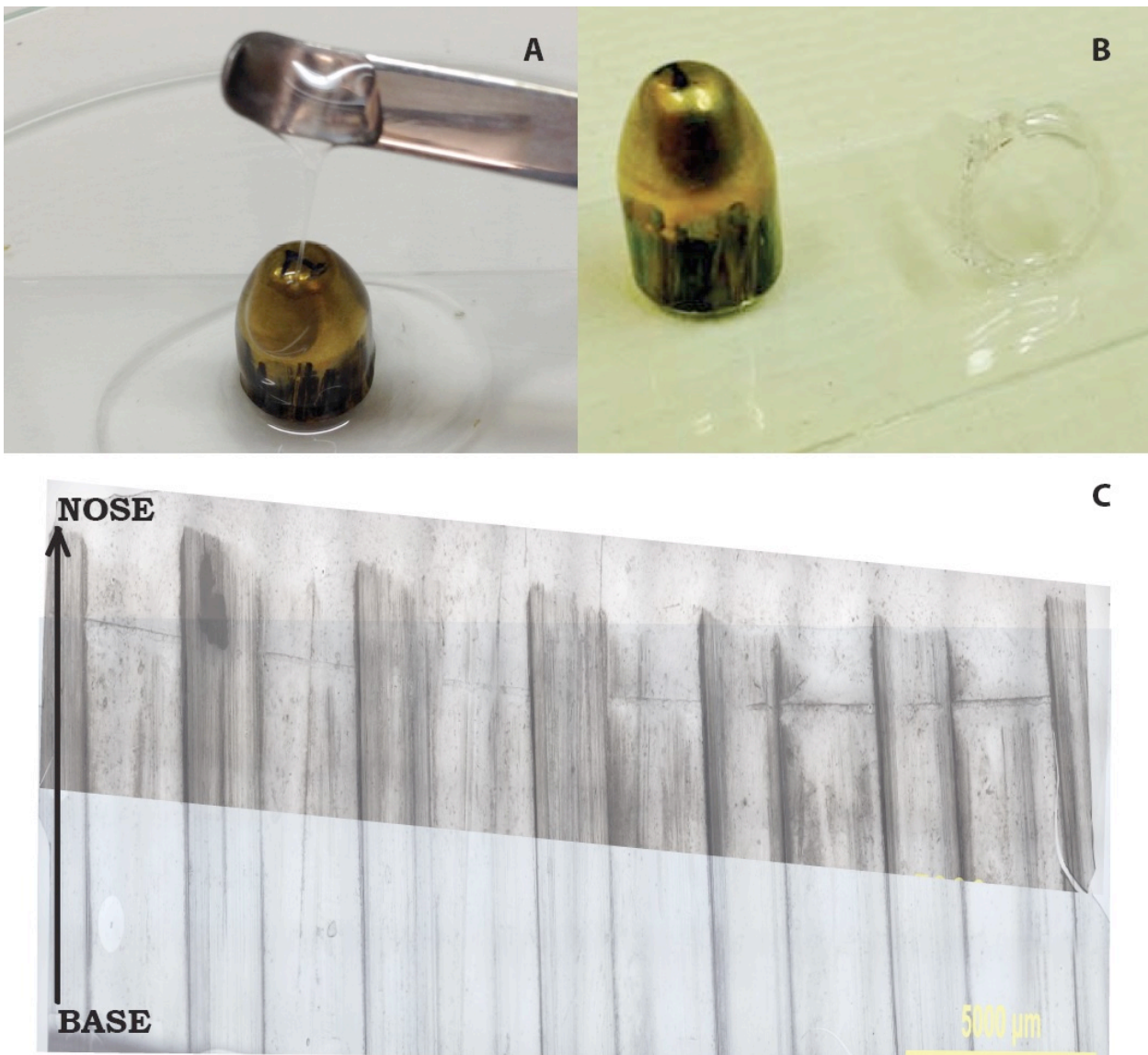
The Power Spectrum Density (PSD) is the square norm of the Fourier transform topographic image, and represents the contribution of each spatial frequency to the topography [17]. Optical profilometry

images were transferred to Gwyddion [18] and one dimensional PSDF were calculated along the direction perpendicular to the striae.

The analysis allows one to explore a range of frequencies  $\nu$  between  $\nu_{\min} = 1/L$  (L being the image size) and the Nyquist frequency  $\nu_{\max} = N/(2L)$  (N being the number of pixels of the image), [19] thus achieving a resolution of less than  $1 \mu\text{m}$ .

### Results and discussion.

Replica molding as a micro or nano-fabrication technique has been widely exploited in the last decades; the range of its applications has varied from the common realization of microfluidic devices [20] to the development of high quality stamps for unconventional fabrication technology [21]. In figure 1 the method for fabricating of the replica of the bullet lateral striae is reported. Part of the experimental procedure can be found in a previously published article by the same group [14] the main differences characterizing the present work are in the requirement of the replica to stand on the lateral surface of the cylinder being at the same time thin enough to be laid on a planar surface.



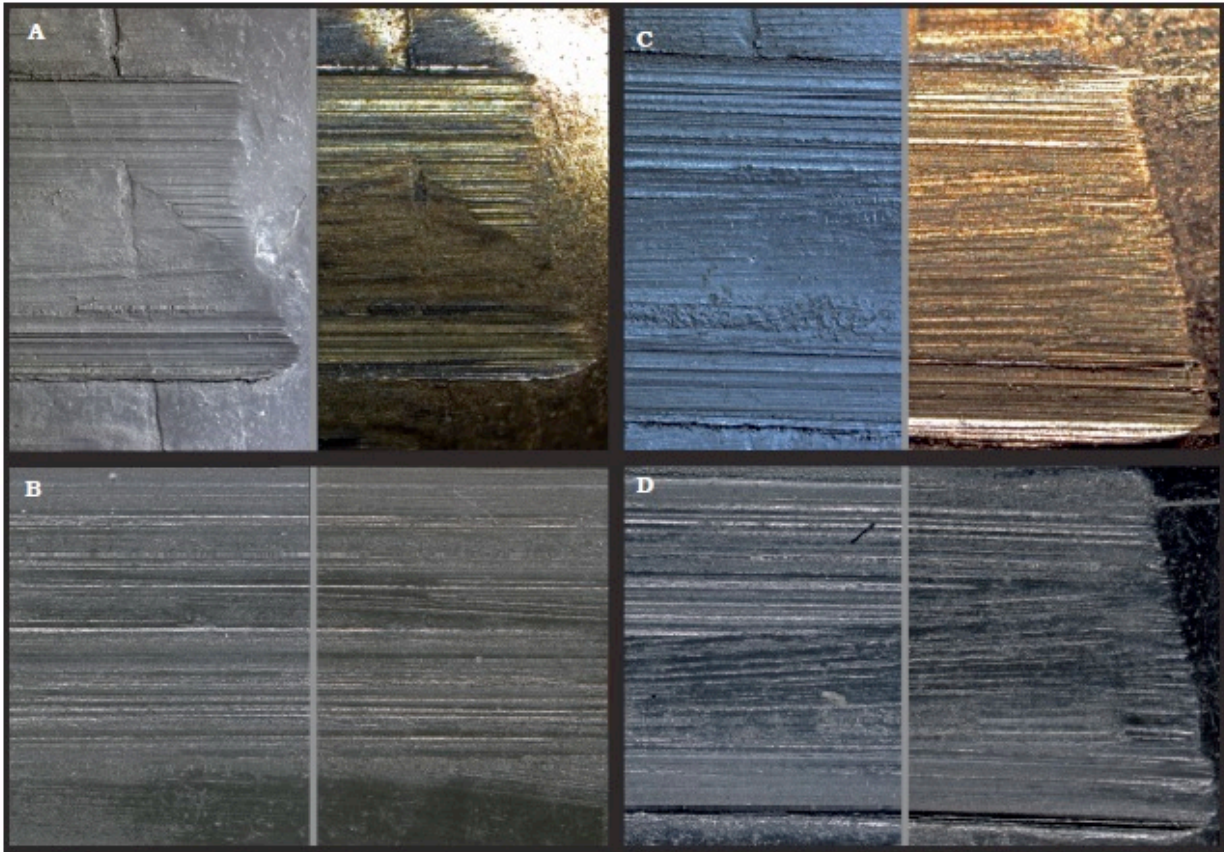
**Figure 1:** Description of the method for fabricating replicas of toolmarks of bullet. In A) the viscous PDMS is poured on the surface to be replicated and upon cross linking a faithful replica B) of the sample is obtained. In C) is shown the partial overlap of two whole replicated profiles of the same bullet after they have been elongated on a plane.

The PDMS is poured (fig 1A) on the vertically standing bullet to achieve a complete coverage of the surface, the ratio of the PDMS:reticulating agent and the temperature for the curing have been tuned for obtaining a homogeneous film. The latter parameter is the most crucial because a too slow curing would cause the slow dripping down of the fluid PDMS thus to a thickening of the lower portion and to a final inhomogeneous sample. The film realized in such a way (fig 1B) will then be laid on a glass slide for its morphological characterization. A possible issue of the PDMS samples is that they can easily adsorb environmental contaminations (powder, micro-hair), thus a further important step consists in sonicating it in 99% ethanol for 5 minutes. This treatment does not damage the replica (PDMS is not brittle at all thus sonication is not eroding it) and promote its good positioning on the underlying support (see supplementary materials).

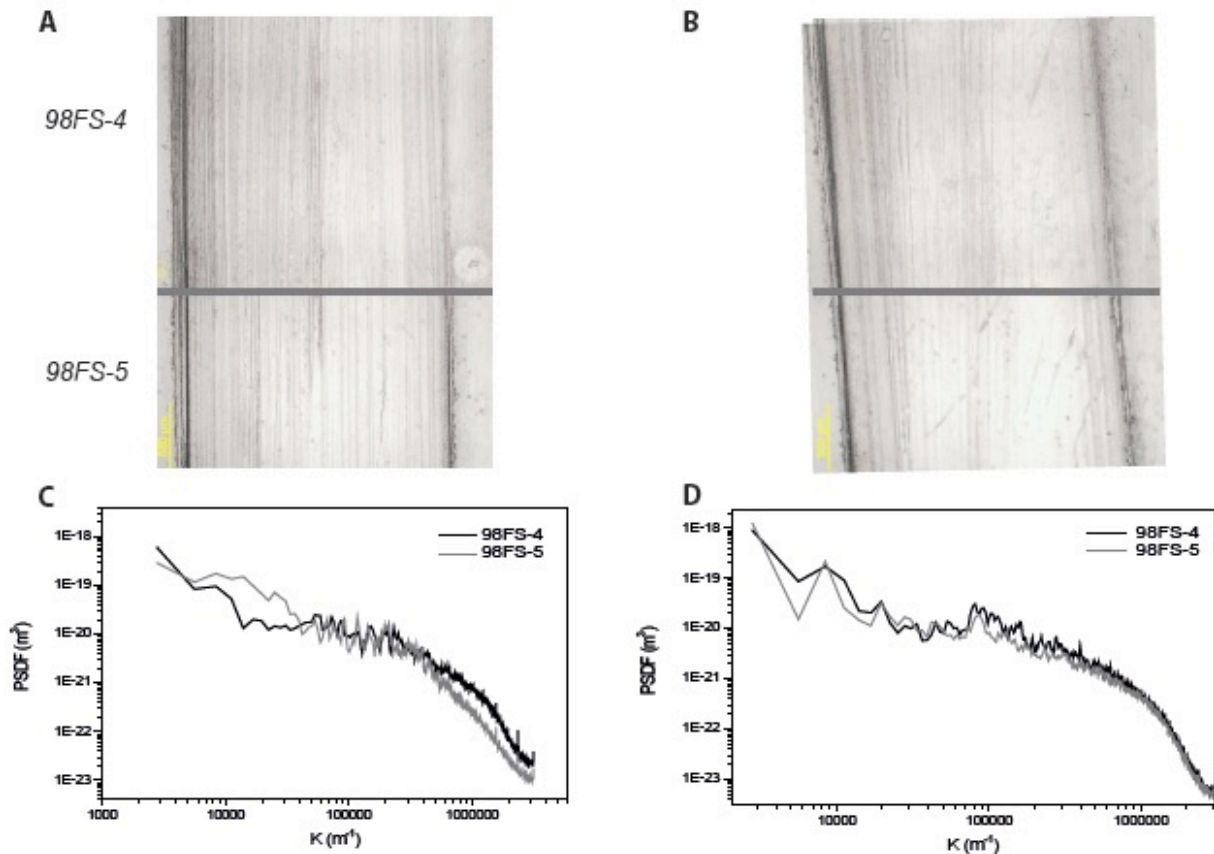
At the end of the described procedures, the user will handle a fully planar sample displaying the whole lateral striae landscape of the chosen bullet. If requested this will allow one to take in single picture the optical image of all the present features because under low magnification they will be in a single focal plane.

Figure 1C is an example of the comparison of two replicas where the whole striae landscape of two bullets can be aligned in a single image. This kind of data allow the direct comparison of characteristics class of the bullet fingerprint and in perspective could prove to be an important tool for a fast and first stage assignment of the class details. It is clear that, at the level of resolution required to appreciate the whole surface, microstriae are barely visible and for their direct comparison higher resolution and magnification are required and they will be reported in the following part to assess the strength of proposed replica method.

We have tested a large group of sample replicas with standard investigation procedures. In figure 2A a first stage assessment of the quality of the replica is reported by mean of the alignment of a portion of the original bullet with the corresponding one on the replica using a standard ballistic microscope; in this way the samples have been also proven to be compatible with classical comparing procedures. Moreover we would like to underline, how a better illumination is achieved on the planar replicas compared to the cylindrical metallic bullet (figure 2A left and right panel respectively). Then two replicas of the same bullet have been compared in figure 2B to show their reproducibility. One can see that the size of the whole groove is the same and by increasing magnification into smaller details the microstriae can be easily aligned, proving that they have been produced by the same weapon. This prove that our replicas satisfy the common requirements of microscopy alignment procedures in the perspective of applying it to real investigations in the conditions where for instance the evidence is held by a different laboratory that will send replicas to the requesting ones for analysis. In such a way the original evidence will not be displaced thus preventing any troubles along the custody chain but the final user will be able to handle a perfect copy for any investigation. The images reported in figure 2C and 2D show a real analytical comparison of evidences where a bullet shot by a Beretta 98FS is compared with the replica of a different bullet shot by the same weapon (2C) and the replicas of two different bullets shot by the same gun are compared (2D).



**Figure 2.** Comparisons of original samples and replicas obtained by a forensic microscope. In A) a single groove of the bullet 98FS-1 is compared to its own replica; in B) corresponding parts of groove of two replicas of the same bullet are aligned to show their reproducibility; in C) the cross-comparison between a replica (on the left 98FS-1\_groove#4) and a bullet (on the right 98FS-2\_groove#4) is reported while in D) in B) two replicas (on the left 98FS-1\_groove#4 and on the right 98FS-2\_groove#4) are compared.



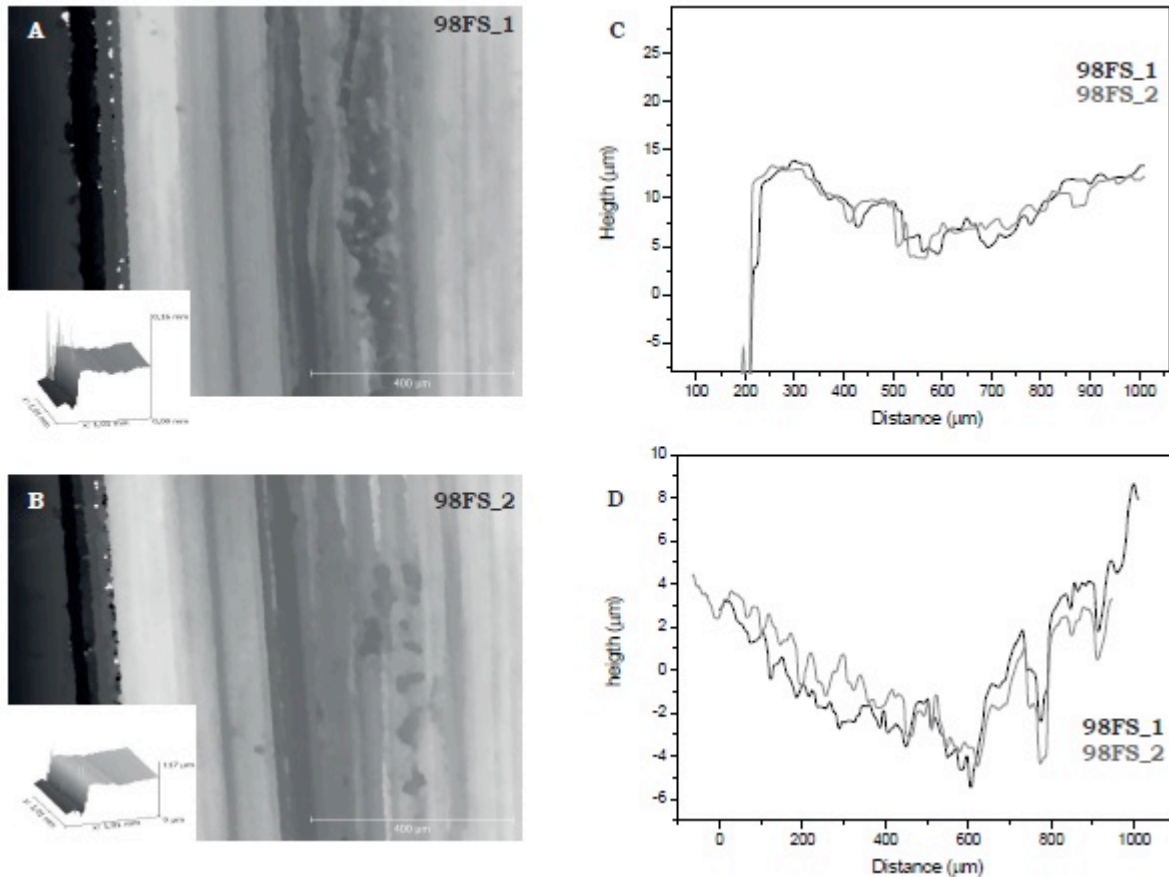
**Figure 3.** Comparison of the lateral side of two different bullets (98FS-4 and 98FS-5) shot by the same pistol (Beretta, model 98FS) at the single groove impression level (micro level comparison). Bright field optical microscopy of two grooves are reported in A) and B) with their corresponding PSDF in C) and D)

To prove advantages arising from the use of planar whole replica of bullet lateral surface, higher magnification optical images obtained with a standard epi-illuminated microscope have been reported in figure 3. Here two corresponding groove areas of two different bullets fired by the same pistol are laterally aligned. Besides the standard alignment procedure further analysis of planar replicas can detect the presence of characteristic lengthscales in these areas. In figure 3C and 3D the Power Spectrum Density Function (PSDF) [22] of the chosen areas are reported; a good description of the information carried by these data can be found in a detailed paper by Biscarini and coworkers [23] and in a previously published paper concerning firing pin comparison [14].

Briefly here one can see that both the slopes, the crossover between different slopes and the peak location can be associated with good accuracy. These parameters describe the presence of different characteristic lengths in the images. It is worth underlining that these kind of analysis is better applied to real three-dimensional topographic image rather than to the two dimensional optical images, but the latter can be most commonly found in a analysis laboratory so we wanted to show that it can provide a first level of association of the whole information included in the images in terms of the presence of periodicities and specific correlation lengths.

To perform a deeper comparison analysis of the features present in the samples that we have fabricated from original bullets a technique providing also information about the real topography is required. The best balance between resolution and versatility is provided by the white light optical profilometry

(WLOP). This technique, based on the interferometry of the light reflected by the sample surface, can achieve in the vertical scale (topography) a resolution of few nanometers, while on the X-Y plane the resolution is the same of optical microscopy. Recently this kind of technology has been also widely applied to forensic ballistic measurements (see the latest models of the Integrated Ballistics Identification System, IBIS, for example).



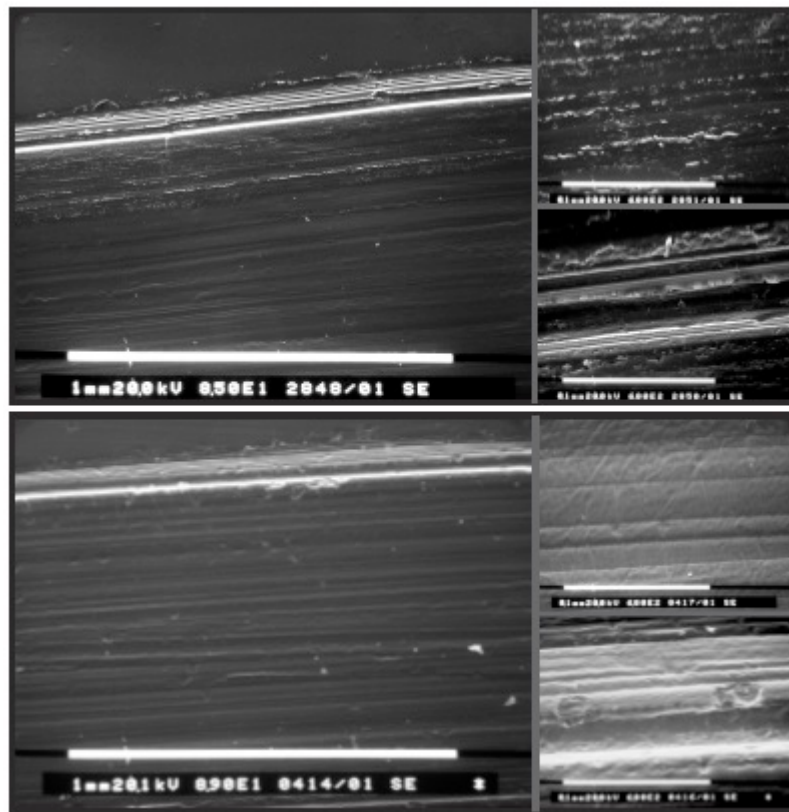
**Figure 4.** White light optical profilometer analysis of the lateral side of two different bullets (98FS-1 and 98FS-2) shot by the same pistol (Beretta, model 98FS). Tridimensional images of two corresponding areas are reported in A) and B); the profiles (98FS-1 dark line, 98FS-2 grey line) resulting from the average of 50 neighbouring sections taken perpendicular to the striae and corresponding to two different position in the images are reported and compared in C) and D).

Compared to a bare optical image, an optical topography prevent all the artifacts due to the illumination issues [10] and allows a further level of comparison of the identified features [24]. As an example in figure 4 two profilometric images of different bullets are reported, the top views are combined with their 3D rendering for appreciating their three dimensional nature. We have here chosen to superimpose a section analysis of the two images along the direction perpendicular to the striae to prove that the lateral colocalization of the features is flanked by the association of their vertical sizes.

The analysis herein reported show the accuracy of the fabrication and comparison of planar replicas from curved ballistic samples.

Nowadays several microscopy techniques allow going to resolutions far larger than those provided by the those reported above. Scanning Electron Microscopy micrographs have been made on two replicas of bullets shot by the same weapons, where one can see at low magnification the sequence of micro-

striae and at high magnification their local morphology [25]. These data show that our method is compatible also with the searching and comparison of very small features on the ballistic samples [26].



**Figure 6.** Scanning electron microscopy images of two replicas of two different bullets shot by the same Beretta 98FS. In the left panels (A and B) the sequence of striae in the nearby of the main groove. In C-F high magnification details of the same areas.

In figure 5 the analysis with Scanning Electron Microscopy (SEM) evidence the nano-details that are proper of the striae and it allows the comparison between replicas together their link to the same gun. This technique does not, in principle, provides any additional information but reports, at very high magnification, the real morfological profile of striae and of some interesting details such as major groove or the micro-periodicity within the features. This is important to outline a strict and unmistakable profile to be associated and to dispel any reasonable doubt as incompatibility. Thus the technique of replica been proven and for made it possible it is real sample release a efficiently nano-copies of bullet analyzable with white light optical microscopy.

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## Conclusions

We have presented a novel method to fabricate planar replicas of the lateral surface of a shot bullet. The advantage of the produced samples is that they display in a straight plane the negative topography of the whole striae landscape preserving their three dimensional nature and just removing the large scale curvature or deformation that is due to the bullet shape (in particular when they have been

subjected to strong impacts). This method proves to be valuable because it allows one to produce an arbitrarily large number of copies of the original ballistic evidence that can be thus preserved from damaging due to manipulation. All these copies can be exchanged between the different law enforcement agencies for their own analysis.

We have shown how these samples allow to overcome some of the drawbacks arising when dealing with original bullets collected on the crime scene; in particular their surface together with their flat shape avoid the artifacts that could be provided by the combination of a far from optimum illumination on a reflective metallic non planar surface and by a non precise sample mounting on the manipulator that can distort the microscopy images. The comparison in a single image of the whole striae sequence displayed by the bullet lateral side allow a very fast first order comparison in terms of their class characteristics (land and groove impressions) that can then be pushed to the individual characteristics (striae) impressed by rifled barrels by simply going to higher magnification images. The PSDF of these images has also been compared to show how the position of their main characteristic lengths can be associated.

Moreover the intrinsic three dimensional nature of these replicas makes them suitable for the most modern topographic investigations as it has been shown by optical profilometry where, besides the lateral size and position of the micro-striae, their 3D shape has been characterized. In this case the profile of the striae sequence has been compared showing that their alignment can be performed also in terms of their topographic features.

The scanning electron microscopy images of the replicas has finally shown how the analysis can be pushed down to the sub-micrometer level.

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