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The *Reed-Stanton* press rig for the generation of reproducible fingermarks: Towards a standardised methodology for fingermark research

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ABSTRACT

In the search for better or new methods/techniques to visualise fingermarks or to analyse them exploiting their chemical content, fingermarks inter-variability may hinder the assessment of the method effectiveness. Variability is due to changes in the chemical composition of the fingermarks between different donors and within the same donor, as well as to differential contact time, pressure and angle. When validating a method or comparing it with existing ones, it is not always possible to account for this type of variability. One way to compensate for these issues is to employ, in the early stages of the method development, a device generating reproducible fingermarks. Here the authors present their take on such device, as well as quantitatively describing its performance and benefits against the manual production of marks. Finally a short application is illustrated for the use of this device, at the method developmental stages, in an emerging area of fingerprinting research concerning the retrieval of chemical intelligence from fingermarks.

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1. Introduction

After over 100 years and despite the advent of DNA technologies, fingerprinting still accounts for most of the identifications in the UK and worldwide [1]. Techniques for visualisation of fingermarks (different from fingerprints which are control prints) have evolved since the 1860s [2] and grown in number including emerging technologies detecting and mapping the chemistry of fingermarks [3]; this indicates an increased keen interest in this type of biometric identification.

However, with an increase of both the number of scientists researching into fingermarks and of fingermark detection and analysis techniques, the necessity to adopt standardised and consistent protocols, when investigating the efficiency and potential implementation of new methods, techniques or technologies, is not only desirable but essential. These protocols would also enable researchers to assess effectiveness, advantages and limitations compared with existing methodologies and a number of standardised tests (test strips or spot tests) have already been proposed as testimony to these needs [4,5], though they

* Corresponding authors. E-mail address: s.francese@shu.ac.uk (S. Francese). are not advised for assessment of operational use but rather for ensuring the reagents are correctly prepared [6]. The issue of the lack of a consistent approach, in the development of existing or new techniques for fingermark detection and analysis amongst the different research groups worldwide, was eloquently described by the Centre of Applied Science and Technology, CAST, Home Office UK in a recent publication [7] that also provided guidelines on minimum standards for scientists undertaking this type of research. This issue was also discussed at the recent International Fingermark Research Group (IFRG) in June 2013 (Israel) and a document has been produced, coordinated by Prof. C Lennard to provide further and more detailed guidance including requirements for publishing the results of the research [8].

One of the major issues, making protocols and techniques not comparable and hindering a valid assessment of a technique's effectiveness, was very well described by Sears and colleagues: "The fundamental issue that needs to be addressed in any assessment of a fingermark enhancement technique is the variability of fingermarks, both between the marks deposited by different people and between marks deposited by the same person over a period of time. If this variability is not taken into account in experiments, then a false impression of the effectiveness of the technique may be created" [7].

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This variability pertains to the chemical nature of the mark (eccrine, groomed, ungroomed), as well as to the contact time, pressure and angle of the individual's fingertip touching a surface to deposit the marks. The necessity to generate reproducible patent marks may introduce further variables that are difficult to control such as chemical composition of the contaminant, amount of the contaminant (e.g. blood, mud, grease, paint) prior to the transfer to the deposition surface and after the transfer to fingertips; this is also very well discussed by Farrugia et al. for the generation of footwear impressions [9]. Fingermark residue depletion is an additional factor to account for when depositing replicate marks; replicate marks are recommended by CAST and are necessary for a reliable interpretation of the results and trends to address fingermark inter-variability. The variable quantity and nature of chemical residue impacts on the evaluation of technique effectiveness because enhancement depends, in many cases, on interaction (or reaction) with chemical targets and on their abundance in the mark. Lack of robust research of the type recommended by Kent [6] could even lead to either some of the fundamental techniques being sidelined for newer techniques, or to newer technique being hastily discarded. The overall "donor effect" has been already highlighted by other researchers as severely hindering a meaningful technique intercomparison and the assessment of the influence of factors such as slight changes in protocols, surfaces and climate, if this is undertaken in different geographic locations [4,10,11]. However whilst the chemical composition variability can, to an extent, be controlled (depositing all the marks at the same time of the day for a standalone experiment that will not be repeated on other days, rubbing fingertips against each other prior to deposition to even the composition, selecting a type of sweat etc.), even replenishing the fingertip with material, by rubbing fingerprints against each other a defined amount of times, in between replicates does not fully address mark deposition variability due to inconsistent contact pressure and contact time which also lead to variability in the amount of deposit transferred.

Furthermore, it is not always possible to obtain a quantitative measure of fingermark inter-variability thus preventing accountability when assessing the effectiveness of the technique employed. One way to circumvent this issue in the first stages of development of a technique is using a device generating reproducible fingermarks such that the fingermark chemistry as well as the first and second levels of ridge detail remains the same throughout the number of replicate samples generated for the specific piece of research undertaken.

Fieldhouse captured very early the impact of the fingermark intervariability issues [12] and published in 2011 [13] the first example of such device, named *fingermark sampler*, enabling the generation of fingermarks under controlled conditions of force applied, contact duration and contact angle during fingermark deposition. Through fingermark grading, following the 0–4 grading scale scheme [14], her work demonstrated consistently high quality in the fingermark deposition across a range of participants and superior reproducibility over "manual deposition".

In the same year, within the Engineering for Life scheme awarded by EPSRC and Sheffield Hallam University, a project was undertaken to engineer a device enabling homogeneous and contactless powdering of latent marks [15] involving an industrial designer, a software engineer, a forensic scientist (in the very early stages) and led by the corresponding author in the capacity of a mass spectrometrist. In order to assess powder homogeneity, fingermark inter-variability had to be taken out of the equation; independently from the work of Fieldhouse (the authors were not aware of this research at the time), another fingerprint generator had been conceptually developed and engineered to generate reproducible fingermarks. This alternative rig, that was named the Reed-Stanton press rig, is an electro-mechanical device comprising a number of custom and OEM parts. Though the rotation/orientation of the finger is managed in a similar way, this rig is configured and controlled to allow independent and variable load/pressure selection and independent setting of contact time. Differently from the Fieldhouse fingermark sampler, the Reed-Stanton press rig allows pressure regulation (as opposed to defined/fixed load (309 g)) as well as regulating the time/ duration of the contact between the fingertip and the deposition surface (to 1/10th second rather than at the discretion of a manual operator) in addition to controlling the contact angle. These factors are controlled/ regulated also when spiking fingertips with any substance before a fingermark is generated. This device and its configuration are reported in Fig. 1. As well as differences in the design, a fundamental difference in the assessment of the quality of the marks produced exists between the press rig described here and the fingermark sampler.

The present paper describes this alternative fingermark generator and its operation, quantitatively demonstrating superior performance against the most attentive manual deposition of fingermark replicates. Finally a brief extract of a larger piece of research is illustrated to describe one of the possible applications of this rig that is the investigation and determination of fingermark age. In the corresponding author's laboratory, Matrix Assisted Laser Desorption Ionisation Mass Spectrometry (MALDI MS), in both profiling and imaging modes, is used to investigate the chemistry of the fingermarks and provide a vast range of forensically relevant information [3], with fingerprint ageing being a very current, highly topical and a much needed area of investigation; accurately placing a suspect at the scene of crime, through the age determination of their fingermarks, would warrant the ability to steer the enquiry in the right direction at the early stages of an investigation as well as proving/disproving the defendant's claims in a court of law. However, this information is still considered the "holy grail" of forensic science; this is probably due to the necessity for very complex and comprehensive studies. These studies need understanding of the research question at a fundamental and molecular level as well as requiring the analysis and cross-reference of a number of environmental and deposition surface factors. For this reason, in preliminary studies, variables need to be minimised in order to gather insights into the feasibility of the technology and of the method being employed for this scope. The use of the Reed-Stanton press rig in this short study presented here indicated a feasible methodological route to investigate and determine fingermark age by showing statistically significant discrimination between fresh, 1, 4 and 8 day old simulated marks.

2. Materials and methods

2.1. Materials

Pre-coated TLC aluminium sheets, ethanol and glass slides were purchased from Sigma-Aldrich (Poole, UK). Latent print reference pads are sold by CrimeTech (http://stores.crimetech.net/latent-print-reference-pad-sebaceous-oil/). Pre-inked fingerprint strips were purchased from Crime Scene Investigation Equipment LTD (www.csiequipment.com). TFA, acetonitrile and $\alpha\text{-cyano}$ 4 hydroxicinnamic acid were purchased from Sigma (Poole, UK). Double sided conductive tape was obtained from TAAB.

The assembly of the press rig comprised a series of laser cut 5 mm thick clear acrylic sheets (Plasticsheets.com), 3D printed polymer components and off the shelf (OEM) componentry such as electromechanical switch gear and linear, 12 V DC, Continuous Duty actuating Push Type solenoids (RS Supplies, http://uk.rs-online.com/web/). A timing control PCB was used to manage fingermark deposition time.

2.2. Methods

2.2.1. Instrumentation and software

The Visual Spectral Comparator (VSC4CX, Foster & Freeman, Evesham, UK) was employed to visualise fingermarks at 254 nm and capture a jpeg image. Image annotation was achieved using Artweaver 3.1.6 (Boris Eyrich Software, Germany). Mass spectrometric Imaging analyses were carried out on a modified Applied Biosystems API Q-Star Pulsar i hybrid Matrix Assisted Laser Desorption Ionisation

H. Reed et al. / Science and Justice xxx (2015) xxx-xxx

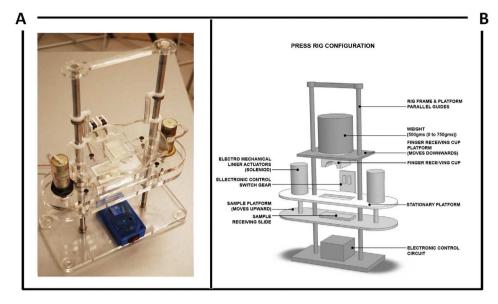


Fig. 1. Second generation press rig device for the production of repeatable fingermarks (the *Reed-Stanton* press rig). Panel A shows the built device whereas a schematic of operation and control is shown in panel B.

(MALDI) quadrupole time-of-flight (QTOF) instrument (Concord, Ontario, Canada). The orthogonal MALDI source has been modified to incorporate a SPOT 10 kHz Nd:YVO4 solid-state laser (Elforlight Ltd., Daventry, UK). Mass spectral imaging data were viewed in Biomap (Novartis, Basel). Principal component analysis (PCA) and partial least squares (PLS) statistical analysis were performed using SIMCA 14 software package (Umetrics, Crewe, UK). All fingermark image analysis was performed using Matlab (Matlab v14a, Mathworks, Natick, MA, USA).

2.2.2. Fingerprint generation

Three sets of inked prints were investigated. In all cases, pre-inked strips were used to produce visible prints and plain white paper was employed as deposition surfaces. Both the white paper and the preinked fingerprint strips were preliminarily cut to the size of a glass microscope slide. Fingerprint sets were as follows: Set 1—a depletion series of 5 "manually deposited fingerprints" and another of 5 fingerprints generated by the press rig were produced to assess consistency in the expected reduction of quality of the print due to the progressive loss of ink from the fingertip; Set 2-4 fingerprints were generated by the press rig at a time interval of 1 week to assess reproducibility of the rig over time; Set 3–10 "manually deposited replicate fingerprints" and 10 replicate fingerprints generated by the Reed-Stanton press rig were produced to assess performance and reproducibility of this device. The index finger to be used for generating control prints was cleaned with a 70:30 Ethanol/H2O solution (Solution A) prior to every replicate deposition for Set 3 of produced fingerprints.

For "manual" deposition of the prints, the operator attempted to reproduce as identical as possible ridge patterns by seemingly applying every time the same contact time, pressure and angle. In particular, the index finger made contact with the pre-inked fingerprint pad for ~3 s (a stopwatch was used) with medium pressure and as perpendicular as possible to the plane of the strip; subsequently the fingertip made contact with plain white paper for another 3 s. This process was repeated for each fingerprint deposition and preceded by washing the fingertip with Solution A to remove residual ink.

For the generation of prints using the *Reed-Stanton* press rig (Fig. 1A), a 3D printed (ABS Thermoplastic) finger receiving 'cup' was manufactured in-house and inserted into the finger receiving cup of the rig to enable slotting of the back of (finger nail side up) the index finger used for fingermark deposition. The template was built ensuring that the top and tip of the finger touched the walls of the template to

allow a reproducible insertion of the finger; this ultimately generated consistency in the fingertip area available to generate the ridge pattern as well as consistency in the fingertip contact angle. The pre-inked fingerprint strip was secured to a glass slide that was then secured to the bottom plate/sample platform of the rig. The press rig power supply was turned on and the manual switch was activated, sending the lower plate upwards to its predetermined position. A 500 g weight was used (ensuring constant quantity of force applied) and then placed onto the top plate, pushing it downwards and allowing the fingertip to make contact with the pre-inked strip for 3 s. After this time, the lower plate dropped away (this ensured constant contact time) and the manual switch was deactivated. This allowed transfer of ink onto the fingertip surface. The pre-inked fingerprint strip was then removed and replaced by a plain white paper strip secured onto a glass slide which was positioned in the slide holder within the lower plate. When the manual switch activated, the lower plate was pushed upwards, whereas the 500 g weight placed onto the top plate allowed contact of the fingertip with the paper surface for 3 s after which the lower plate dropped away carrying an inked fingerprint on paper. This process was repeated for each print deposition. A schematic of operation and control of the press rig (second generation device) is given in Fig. 2 and shown in the video provided in the Supplementary information.

2.2.2.1. Assessment of fingermark quality. The quality control of the prints generated "manually" and by the *Reed-Stanton* press rig was undertaken by two different means namely a) 0–4 fingerprint grading [14] and b) fingermark image analysis. In particular method a) was applied to all of the three fingerprint sets, whereas method b) was applied to *Set* 3 only as proof of concept.

With method a) all of the clear *minutiae* were marked up and some fixed quality markers were used to examine how consistent the area of recording was. The quality markers chosen were located close to the core and delta of each impression and the consistency and quality of the recorded area were judged using the following analyses: 1. A horizontal line was taken from the ridge ending (shown in yellow in Fig. S1 displayed as an example) located directly above the delta to the right hand side of the print. The number of ridges intersecting this line was then counted to give a 'ridge count' towards the left hand side of the impression; 2. A horizontal line was taken from the ridge ending (shown in red in Fig. S1) directly above the core to the left hand side of the image to give a ridge count to the right hand side of the impression; 3. A vertical line was taken from the same ridge ending

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H. Reed et al. / Science and Justice xxx (2015) xxx-xxx

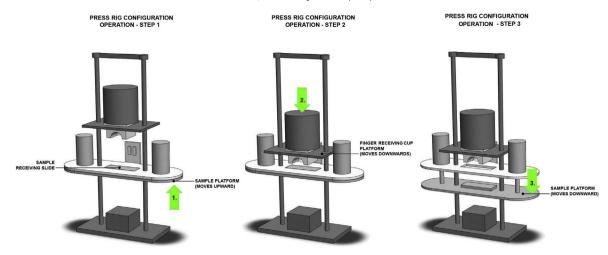


Fig. 2. Three step sequence for the generation of reproducible fingermarks using the *Reed-Stanton* press rig. First the sample platform is moved upward, then the finger receiving cup platform is moved downwards and finally the sample platform is returned to its original position (downward movement).

(above the core of the pattern, shown in red) to give a ridge count to the top of the impression; 4; Marks were finally also graded according to the 0-4 grading scheme [14]. With method b) images of the fingermarks (obtained through UV-vis image capture) were analysed using computer vision techniques that were independent of the position, orientation and focal length of the camera used to obtain the images. UV Imaging was used to exploit the chemical structure of the ink absorbing UV light and thus permitting the retrieval of images even at reduced ink quantities in depletion images. To assess the repeatability of a method, each of the 10 images was compared to each of the others in a pairwise manner. The images were initially processed to identify usable image features — the silhouette and the ridges of the fingerprint. Silhouette Images — Silhouette images were generated through a process depicted in Fig. 3A. The colour image was converted to greyscale before applying morphological operations to smooth the object and background colour regions. This smoothed image was converted to a binary image using a threshold of 0.5 on the pixel intensities (on the scale of 0-1). The compliment is taken so that the white silhouette represents the fingerprint area rather than the background. Another morphological operation was applied to remove noise from the binary image. Ridge images-Ridge images were generated through a process depicted in Fig. 3B. The silhouette image was used as a mask to remove the background. The image was then converted to a greyscale image. The greyscale image was converted to a binary image using a threshold value that was determined from the pixels in the image using the method described by Otsu [16]. The threshold is adjusted if less than 25% of the pixels are set to white. This binary ridge image shows where the ink was absent within the fingerprint—("negative" image). This is a better image to fit than using the regions where the ink was present because there are large regions of continuous ink in some prints within the lower middle area, with Fig. 3A–B being an example of this.

An initial comparison was carried out using the silhouette images (SI), SI₁ and SI₂. They are moved and rotated to maximise the proportion of the white regions that overlap. This is equivalent to maximising the purple region shown in Fig. 4A. The resolution of the search pattern used to match the silhouette images was 1 pixel and 1°. The proportion of the purple region in Fig. 4A(ii) as a percentage of the mean size of the silhouette images is used as a measure of how well the general shape of the two fingerprints matches. This is denoted the 'match index'. With respect to ridge images, two ridge images (RI), RI₁ and RI₂, are compared using two-dimensional correlation for a range of rotation and scale values. For each combination of rotation and scale, ridge image RI₂ is rotated and scaled as such to give image RI₂' and image RI₁ is inversely scaled and rotated to give image RI₁'. Image RI₁ is correlated with Image RI₂' to give the accumulator matrix M1 and Image RI₁' is correlated with Image RI₂ to give the accumulator matrix M2. M2 is rotated 180° to match the orientation of the original images and added to M1 to give M. The accumulator matrices are illustrated in Fig. 4B. The position

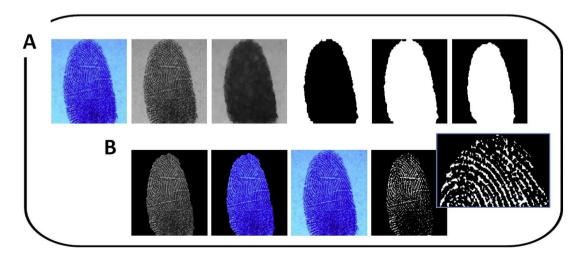


Fig. 3. Optical image conversion to binary images. Panel A shows an example of the image processing from the original image acquired to binary finger silhouette; panel B shows an example of the process from the initial image to binary ridge image. The inset in the blue frame shows a close-up of the ridge image in Fig. 2 to illustrate the noise which can clearly be seen between ridges and the discontinuous nature of some of the ridges.

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H. Reed et al. / Science and Justice xxx (2015) xxx-xxx

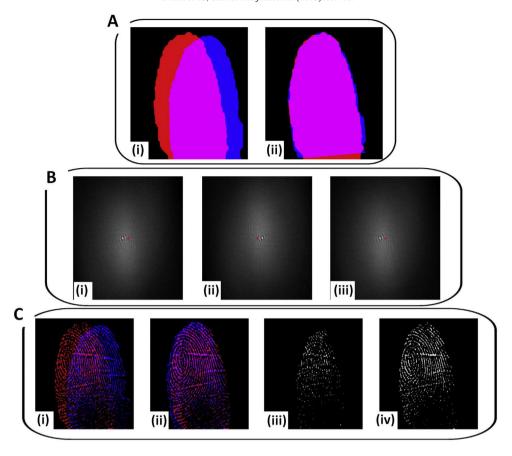


Fig. 4. Silhouette and ridge images comparison (Set 3 of fingerprints). Panel A shows the overlay of silhouette image 1 (blue) on silhouette image 2 (red) with the overlap shown in purple, (i) before and (ii) after the silhouettes have been fitted. Panel B shows: the accumulator matrices (i) M1 from image (a) and image (b'), (ii) M2 from image (a') and image (b) and (iii) M, the sum of M1 and M2 (rotated by 180°). The centre is marked in red for reference. Finally panel C illustrates the overlay of ridge image (b) (blue) on ridge image (a) (red) with the overlap shown in purple, (i) before and (ii) after the ridges have been best matched. The purple regions are shown as white in (iii) and (iv).

of the maximum value in M relative to the image centre indicates the movement required to best match image RI2 to image RI1 for that given rotation and scale. The highest maximum value of M through all of the ranges of rotations and scales shows the best match. The rotation, scale and translation from the best match are accepted as the adjustment applied to image RI₂ to best match image RI₁. The resolution was 1 pixel for movement, 0.25° for rotation and 0.25% for scale. An example of before and after the best match is found is shown in Fig. 4C. The guality of the match before and after can be seen in Fig. 4C (iv); the ridges are continuous, the horizontal crease lines are clear and the centre of the whorl pattern (the core) is clear. None of these would be the case if the fingerprints were not well matched, as in Fig. 4C (iii). Similar to silhouette images, the match index was defined as the proportion of matching pixels after the best match is found (Fig. 4C (iv)) as a percentage of the mean number of pixels in images RI₁ and RI₂. For reference, a large proportion of the pixels will be noise, as seen in Fig. 3A. Also, some of the ridges are not continuously identified in images RI₁ and RI₂. Where they are identified in RI_1 , but not in RI_2 – or vice versa – these pixels will count against the match index. As such, a ridge match index of more than 40% indicates a good match. When fingerprints that are not from the same finger are compared, match indices of less than 40% are found.

2.2.2.2. Fingerprint ageing using the Reed-Stanton press rig. "Flat fingermarks" were employed for this study. These are flat and linear patterns produced by a silicone master stamp in the press rig. The marks were generated using a latent print reference pad containing sebaceous oil secretions. One stamp is used and it is cleaned with ethanol between each sample to minimise even the smallest variability between samples using the silicon 3D fingertips. The lipid material is lifted

and then deposited on aluminium slides (see video provided in Supplementary information showing preparation of these marks). Three replicate fingermarks were collected at four ageing time points, namely 0, 3, 4 and 8 days. The 0 day time point refers to fresh fingermarks which were prepared and analysed immediately after deposition. The other time points refer to marks generated and stored at constant 37 °C with 5% CO₂ in a cell incubator. Fresh and aged marks were coated with α -cyano 4 hydroxycinnamic acid matrix powder. The powder was deployed using a proof of concept gun developed under the Engineering for Life scheme (UK patent application GB 2504276) and currently under further industrial development. The gun provided uniform matrix coating. Marks were subsequently uniformly spray coated in a 70/30 Acetonitrile/TFA0.1% solution using an automated sprayer SunCollect autosprayer (SunchromGmbH, Friedrichsdorf, Germany), at a flow rate of 5 mL/min and using "fast raster" setting. Marks were then analysed by Matrix Assisted Laser Desorption Ionisation Mass Spectrometry Imaging on a modified Applied Biosystems API Q-Star Pulsar i hybrid quadrupole time-of-flight (QTOF) instrument (Concord, Ontario, Canada) in positive ion mode and in the mass range 50-1000 Da as previously described [17]. Data were viewed in Biomap (Novartis, Basel); a mass spectrum for each fingermark was obtained by averaging acquired spectra across the whole fingermark area. Averaged spectra were processed using principal component multivariate analysis.

3. Results and discussion

In this work, the *Reed-Stanton* press rig, an alternative "fingermark generator" to that proposed by Fieldhouse in 2011 [13], is illustrated. The performance of this sampler has been described using a 0–4 grading

Table 1Fingermark grading and quality assessment for a depletion series of manually deposited and *Reed-Stanton* press rig deposited prints (*Set 1*).

Manual deposition Press rig deposition Number of Grade Grade Impression number Number of characteristics characteristics 3 49 4 2 48 3 33 3 3 3 32 3 26 4 15 2 23 3 11 6 2.6 24.4 2.6 31 Average Standard Deviation 17.82 1 14 11 28 0.89

[14]. Additionally and differently from Fieldhouse's work [13], performance was also quantitatively described, thus more robustly supporting the benefits of such device in the early assessment stages of a new method/technique for fingermark visualisation and or/analysis of the chemical content. In the Fieldhouse's device, no variable control is described pertaining the force to be applied and contact time is at discretion of manual operator. The Reed-Stanton press rig configuration allows definition of the contact pressure (10 or 750 g for example), selectable contact time and contact angle. The pressure/angle/time parameters used for depositing the marks are the same employed for spiking fingertips (for touch chemistry studies) thus allowing more repeatable conditions across the whole experiment. Inked prints were used for the study. Whilst it can be argued that the employment of currently used enhancement techniques will have to be used in the near future for a more comprehensive assessment of the device, for this initial proof of concept, inked marks were deemed to be fit for purpose. In the studies illustrated here, the "manual" deposition of inked prints was performed by taking much care with regard to the above variables and overall attempting a reproducible generation of fingermarks; this allowed a more challenging (but fairer) and less obvious comparative assessment between fingermark generated "manually" and through a semi-automatic device such as the press rig. To evaluate and compare the quality of the prints generated through either manual deposition or the press rig, or captured through UV imaging, two processing methods were used. In the first method (method a), all of the clear characteristics have been marked up in addition to using fixed markers to check consistency of the recording area (as described in Section 2.2.2.1). Each image was analysed with all of the clear minutiae being annotated. In terms of the characteristics recorded, for Set 1 of fingerprints (Fig. S1 A), the reduction in the number of characteristics that was seen through each sampling method is as expected for a depletion series, with the press rig showing a more consistent and gradual decline in the quality of fingermark observed. However, even though the Reed-Stanton rig generated impressions with a more consistent and gradual decrease in the ridge intensity, the rig fingerprint data set displays a reduced number of characteristics overall than the "manually" deposited prints (49, 48, 32, 15, 11 against 34, 33, 26, 23, 6 for the manual deposition and rig deposition respectively), mainly due to an area of distortion that can be seen in the lower third of the print. This may have been due to the finger not being introduced exactly perpendicular to the plane of deposition (a similar issue is seen in the "over time set" (Set 2), but this was subsequently rectified). These data are summarised in Table 1.

In terms of the reproducibility of the depletion series, the number of characteristics seen (albeit reduced in number compared with the manually deposited marks) and the quality of the print deposited show good consistency across the press rig series in the expected reduction of the quality of the print, due to the progressive loss of ink expected in a depletion series. The manually deposited series is also consistent across all measurements. The only exception for both sets is for image 5 of the depletion, where a dramatic decrease in both number of characteristics and the quality of the print is seen, but this loss of clarity is most likely

Table 2Fingerprint grading and quality assessment for a deposition series over time (*Set 2*), using the *Reed-Stanton* press rig.

Deposition period	Press rig deposition						
	Number of	Grade	Ridge count:				
	characteristics		Core to top	Core to LHS	Delta to RHS		
Week 1	62	4	14	8	3		
Week 2	52	4	14	6	5		
Week 3	56	4	15	7	3		
Week 4	56	4	17	8	4		
Average	56.5	4	15	7.25	3.75		
Standard deviation	3.57	0	1.41	0.96	0.96		

due to their position in the depletion series and so should not be seen as detrimental to the data.

As a result of the rig adjustment, made in response to the lower third distortion seen in *Set 1* (see above), the series of 4 images taken over time using the press rig (*Set 2*) display much better clarity and show a superior number of characteristics (62, 52, 56, 56), compared to the manually deposited prints in the previous data sets (Fig. S2 B). The number of characteristics seen and the relative grades of the fingermarks are shown in Table 2, where data is reported for the press rig only as the aim was not a comparison with a manually deposited set but the absolute assessment of the performance of this device over time.

The fingerprints obtained via the *Reed-Stanton* press rig in the *Set 2* (Table 2) showed high levels of consistency, with minimal variation being seen with regards to the various counts and a higher average number of characteristics being seen overall when compared with the manually deposited prints collected in other sets.

The grade of the fingerprints was also consistently high, with all five fingerprints having full ridge development (grade 4). The only potential issue raised in the analysis was with the print deposited in week 2. This showed a greater variation in values compared to the other three prints and this is thought to be due to a slight change in angle of deposition. This is being further investigated and it is hoped that a small alteration to the way the finger is placed into the receiving 'cup' may resolve this issue. Currently, a finger is in contact with the 'cup' at the tip and on the back of the finger (nail side). This may still allow for some slight rotational movement. If the finger is also made to come into contact with a surface on the left or right hand side of the 'cup' then this may further stabilise the finger and ensure a consistent lateral angle of deposition.

The final data set analysed (*Set 3*) further highlighted the consistency and improved quality of results obtained using the *Reed-Stanton* press rig (Fig. S3). The fingermark quality assessment results are displayed in Table 3.

The manually deposited prints in this Set highlighted the importance of control of deposition pressure, as the upper areas of these manually deposited prints repeatedly suffered from broadening and flattening of ridges due to (most likely) increased deposition pressure in these areas. This caused the prints to be of very poor quality in some areas and prevented accurate ridge count analysis (when assessing the repeatability in terms of surface area recorded) as the ridges were not clearly defined. This is similar to the issue that was initially seen in the lower third of the deposition series of press rig prints in Set 1, the crucial difference being that alterations could subsequently be made to the rig to reduce and/or rectify the issues; no such adjustments can reliably be made when depositing prints manually, highlighting a significant advantage of using the press rig with regards to consistency of deposition pressure. Set 3 was specifically generated to assess the reproducibility of results over a series of fingermark depositions. The results demonstrated outstanding consistency in this respect. Despite care being taken to produce repeatable results via manual deposition, the repeatability of fingermarks produced using the press rig was far superior.

Table 3Fingermark grading and quality assessment for a repetition series of manually deposited and *Reed-Stanton* press rig deposited impressions (*Set 3*) (Note: values shown in grey boxes and in italics are estimations, as it was not possible to obtain an accurate value due to the poor quality of the print in some areas).

	Manual deposition				Press rig deposition					
			Ridge count:				Ridge count:			
Image no.	No. of character	Grade	Core to top	Core to LHS	Delta to RHS	No. of character	Grade	Core to top	Core to LHS	Delta to RHS
1	25	3	19	10	1	48	4	12	6	4
2	24	3	19	9	3	37	3	10	5	4
3	25	3	20	9	2	32	4	11	5	4
4	40	3	20	8	4	34	3	10	5	4
5	21	2	18	8	3	36	3	10	5	4
6	28	2	18	8	3	33	3	10	4	4
7	32	3	18	6	4	30	3	10	5	4
8	39	4	19	8	4	38	3	10	5	4
9	49	4	19	9	3	38	3	10	5	4
10	18	2	18	7	4	33	3	10	4	5
Aver.	30.1	2.9	18.8	8.2	3.1	35.9	3.2	10.3	4.9	4.1
Standard dev.	9.78	0.74	0.79	1.14	0.99	5.02	0.42	0.67	0.57	0.32

Not only did the manually deposited prints suffer from large areas of poor quality ridges (most likely due to increased deposition pressure), they also showed greater variability in terms of the area of friction ridge detail that was recorded. Although some variation in terms of area captured may be expected due to the elastic nature of the skin, fingermarks recorded using the *Reed-Stanton* press rig showed a marked reduction in variability of ridge counts. The superior quality of the *Reed-Stanton* press rig was also reflected in the grading of the fingermarks, with impressions consistently achieving grades of 3 or above (where at least two thirds of the mark contains continuous ridges).

Across all three data sets analyses using method a), the variability (standard deviation) of all values was greatly improved by utilising the *Reed-Stanton* press rig, indicating that the rig deposition would provide more consistent and reliable results than manual deposition when assessing quality of deposited fingerprints and any related development or detection techniques.

A second method (method b) was used to assess reproducibility of fingermarks and compare the performance of the *Reed-Stanton* press rig with manual deposition. This method was applied as an example to the *fingerprint Set 3* and consisted in analysing binary silhouette and ridge images as described in Section 2.2.2.2.

Comparative analyses have shown that there is a statistically significant difference in the match indices between the methods for both the silhouette and ridge image comparisons (Table 2). The effect sizes for both indices are also large but the ridge match index was improved by the *Reed-Stanton* press rig to a greater extent than the silhouette match index. This highlights that the press rig is particularly more reproducible than manual methods in the analysis of ridge detail rather than simply a more reproducible general shape of the mark. In essence,

Table 4The mean and standard deviation of the pairwise match indices, the associated *p*-value between methods and the effect size.

	Standard		Press rig			
	Mean	SD	Mean	SD	p	Effect size
Sillouette match index Ridge match index	95.57 37.09	1.92 2.91	97.00 50.02	1.07 2.80	<0.001 <0.001	0.92 4.52

the image processing technique quantitatively demonstrates that the press rig generates fingermarks that have more consistent features than those manually deposited thus providing a more repeatable means for fingermark generation (Table 4).

Encouraged by the performance of the *Reed-Stanton* press rig, the device was employed in a short proof of concept study (part of a bigger research) to demonstrate its usefulness in the development of methodologies in fingerprint research. In particular, this rig was employed for gaining insights into possible methodologies to determine the age of fingermarks. This is an exciting prospect in forensic science as temporally placing suspects at the crime scene may help in proving/disproving legitimate access as well as the defendant's statement. Before validating any methodology, a proof of concept modelling study is always a good starting point.

Therefore silicon stamped "fingermarks" were employed instead of real fingermarks and were generated by picking up lipids from a lipid pad (spiking). The lipid pad composition was preliminarily investigated by mass measurements and found to contain mainly fatty acids, diacylglycerols and triacylglycerols, which are reported to be contained in real fingermarks. Here the absolute identification of these species was less important as lipid mass spectral data were used in their entirety over the mass range 50-100 Da for subsequent multivariate analysis; however tentative identifications were made and reported in Table S1 using LipidMaps (http://www.lipidmaps.org/). Zero, 1, 4 and 8 days old silicon fingermarks were generated, prepared and analysed as described in Section 2.2.2.2. Mass spectral images were acquired and an average spectrum extracted for each mark. This dataset was preliminarily processed by PCA analysis, where the different ageing times clustered and were separated using three principal components. The data were then analysed with PLS regression where the age information was used as a part of the model (Fig. 5). The analysis was able to create a linear model with $R^2 = 0.989901$, which indicates a fairly good representation of the data.

Zadnik et al. [18] reported on the inadequacy of fingermark simulants such as the commercially available reference pads to assess performance of fingermark enhancement techniques. This is understandable given that they are an incomplete representation of latent marks and are therefore insufficient for the accurate evaluation of

H. Reed et al. / Science and Justice xxx (2015) xxx-xxx

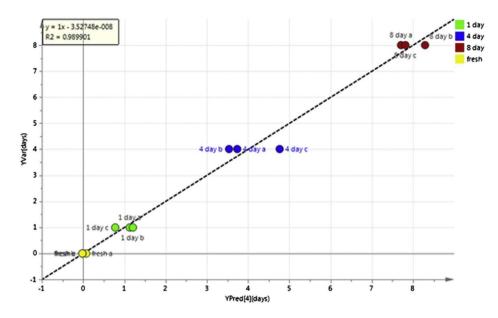


Fig. 5. PLS regression analysis of the MS data for zero, 1, 4, and 8 days silicon model fingermarks.

enhancement techniques across the range (which target sometimes multiple classes of chemicals). Therefore an alternative in this study could have been the in-house making of a solution of a range of molecules and electrolytes known to be contained in sweat. However, this would have been unnecessarily costly for the purpose of this initial ageing study. Also, the possible compositional inhomogeneity of the pad did not prevent statistically significant grouping and discrimination of the age points; this suggests that this possible further limitation did not negatively impact on the performance of the method, that actually demonstrated to be resilient to possible compositional variability of the lipid pad.

The study presented is clearly only a very small step towards the accomplishment of a much bigger research programme that could give an operational outcome, in the view of the authors, only realistically in 10–15 years. In fact, natural secretions are the ultimate target to investigate; though employing them at this initial stage would have introduced too many variables in one experiment making the initial assessment of the quality and feasibility of the method extremely challenging; environmental conditions and deposition surfaces also need to be thoroughly investigated and parameterised to generate a robust model. Furthermore, compatibility with a range of enhancement techniques must also be achieved for the method to become operational. Despite the complexity and the necessary length of this research, to the authors' knowledge, this is the first study, albeit short, demonstrating promise of a MALDI MSI based approach utilising the detection of lipids and a statistical approach to pinpoint the age of fingermarks. The use of the rig was very important to help in a clearer assessment of the potential of this method, though additional time and data points for each of the time points (as well as the use of natural fingermarks) are needed to generate a more robust and close to reality model, posing the basis to determine the age of unknown samples in the future.

4. Conclusions

Given the importance of using reproducible fingerprints for the initial developmental stages of any physical, chemical or analytical methodology, the research presented here has described and proposed the use of fingerprint rig generator (the *Reed-Stanton press rig*), alternative to the only other existing sampler by Sarah Fieldhouse. The definition of the pressure (10 or 750 g for example), angle as well as selectable contact time in the device described here, naturally increases the level of reproducibility in the generation of fingerprints as variable control and selection of parameters can be used to fine tune, set and repeatedly

reproduce fingermarks appropriate to sampling conditions and test criteria. As this is a preliminary investigation of the new device, there are limitations in terms of the full assessment of its versatility and applicability. Future work (part of it is currently in progress) will include (i) the use of a number of current enhancement techniques for the visualisation of marks, as opposed to inked marks, to assess feasibility of the device and (ii) the generation of blood marks to expand investigations on the versatility of the device. The use of in-house standard solutions mimicking eccrine, ungroomed, groomed and importantly natural sweat secretions, will also be employed for a more in-depth and accurate assessment of the enhancement techniques under investigation. These adjustments will be important to inform a subsequent stage of the development of this device in trials that could be undertaken by relevant R&D personnel at CAST and Police scientific labs.

In the present study, both classical fingermark grading and the novel image processing method reported here have unequivocally assessed significantly superior performance on the rig over manual deposition as expected. Furthermore, the use of image processing metrics, enables performance of the device to be quantitatively assessed and outcome assessed against classic fingermark grading for both manually and rig generated fingermarks. An advantage of this quality assessment method is that it is considerable less time consuming than the classic grading system. Furthermore the quantitative nature of the fingermark image processing has posed the basis for further and focussed improvement of the present device. Based on the results of these studies and on the identification of the value of such a device in lab based R&D and evaluation, investment in a third generation press rig has been undertaken and it is currently being trialled with the aim to provide laboratories in need with this rig. Finally, the short proof of concept experiment illustrated here indicates that the combined used of MALDI MSI of lipids and statistical analysis from reproducible fingermark is a promising strategy to investigate and eventually outline a methodology for fingermark dating.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scijus.2015.10.001. The underlying research data are openly available from the Sheffield Hallam University Research Data Archive at http://doi.org/10.17032/shu-150007.

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H. Reed et al. / Science and Justice xxx (2015) xxx-xxx

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9