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The influence of contact force on forensic trace collection efficiency when sampling textiles with adhesive tape

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ABSTRACT

Purpose: DNA is a highly valuable lead to identify people who were possibly involved in a crime. Even by small contact events, minute amounts of DNA ('trace DNA') can be transferred from a DNA source to an evidentiary item, which can be enough for a successful DNA analysis. The focus of this research is to get more insight in the collection of trace DNA from textiles by 'stubbing', which is a tape-lifting method using double-sided tape placed on a stub. The relation between the 'stubbing force' (the normal force that is applied during stubbing) and the collection efficiency of microspheres is investigated.
Methods: Microspheres (Ø25 μm) were used as mock traces to mimic DNA-containing micro-traces. The particles were applied to textile substrates in a suspension of ethanol that was left to evaporate before sampling. Experiments were performed on three different polyester substrates. Traces were collected by stubbing while using 5 different stubbing forces. The number of microspheres placed on each substrate was counted before sampling and all stub-tapes were analysed after sampling to count how many of the microspheres were picked up, both by using stitched images from a digital light microscope. Custom-made image recognition software was used to automatically count the microspheres.
Results: On all tested polyester substrates, the mean efficiency of the collection of microspheres increased with increasing stubbing force in a concave down increasing function. The increase of collection efficiency stagnated around 3–12 N, depending on the substrate material. The theoretical maximum collection efficiencies varied between 38% and 78%, depending on substrate material as well.
Conclusions: Stubbing with a force higher than 12 N does not notably influence the collection efficiency from the variety of textiles that were tested. However, because the theoretical maxima of the collection efficiencies were far from 100%, it is highly likely that stubbing multiple times on the same spot of a substrate increases the total collection efficiency. The gained knowledge will help to standardize and improve the effectiveness of stubbing.

1. Introduction

DNA traces are frequently recovered from evidentiary items, because it is a highly valuable lead to identify people who were possibly involved in a crime. Even from minute amounts of DNA a sample can be obtained from which interpretable DNA profiles can be derived.

When an evidentiary item is sampled, the ideal sample contains as much as possible targeted DNA and as little as possible noise (i.e.: other contaminating material) as to maximize the chance of obtaining a useful DNA profile. The ratio between targeted DNA and noise in the sample is determined by all steps in the DNA transfer process (see Fig. 1). The amount of DNA that humans (the **DNA sources**) transfer to their surroundings (known as 'shedding') varies between persons, but also within persons over time [1]. This is influenced by, for example, hand washing [2] or previous touch events [3]. The **activity** by which DNA is transferred indicates the contact event. In transfer of DNA between different substrates it has been shown that friction contacts transfer much more DNA than passive or pressured contacts [4–6]. **Ageing** indicates the degradation of DNA, which depends on various factors, including time, temperature, humidity, ultra-violet light, exposure to various chemical substances and other environmental factors [7,8]. The substrate of the **evidentiary item** affects the deposition and

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Fig. 1. DNA transfer from a DNA source to a sample from which DNA can be extracted to create a DNA profile.

collection efficiencies of micro-traces on and from it. Besides, specific substrate materials may interact with and degrade DNA [9]. For **sampling** a DNA-containing trace, different methods exist, such as cutting, swabbing and tape-lifting. Tape-lifting has been compared to other recovery methods and was shown to be an easy-to-use and reliable DNA collection method for sampling micro-traces from textiles [10–12].

Of all 5 mentioned factors in the DNA transfer process from the source to the secured DNA sample, sampling is the only step that is controllable by the forensic investigator at the crime scene. Therefore it is quintessential to understand the working principle of a sampling method well in order to achieve optimal DNA collection.

This study focusses on 'stubbing'; a tape-lifting method using a stub covered with double-sided adhesive tape to lift micro-traces from a substrate (Fig. 2). This is an easy to use and reliable method [10] that is mostly used for the collection of micro-particles from textile. Sampling from textile evidentiary items, such as clothing, involves extra challenges as compared to solid objects, because textile is porous and highly deformable. Due to the shape and size of the stubs, stubbing offers much potential for further standardisation of sampling micro-traces. Furthermore, the stub holder creates a distance between the sampled surface and the hand in order to reduce contamination risks. It should be noted that, just as with other adhesive tape based methods, stubbing is less or not suitable for textiles that are wet (no adhesive bonding), that have so extremely been exposed to the elements that they fall apart (textile and traces both getting lifted), or are covered in dirt (sample will contain mostly dirt, which could of course be a trace itself).

Even though various collection methods have been compared with each other [10–14], only a few studies considered the effect of variations within the stubbing technique on the final sample: the effect of tape brand [9,15] and the number of tape-liftings [9] have been studied. In the stubbing procedure that is currently used at our forensic institute, the 'stubbing force' (i.e.: maximum normal force applied on the stub during trace collection) is applied manually, which makes it hard to control. However, the stubbing force is suspected to be one of the most influential variables during stubbing, based on the following working principle. Under a higher stubbing force, textile fibres and tape are compressed more, which increases the actual contact area between the tape and the textile.

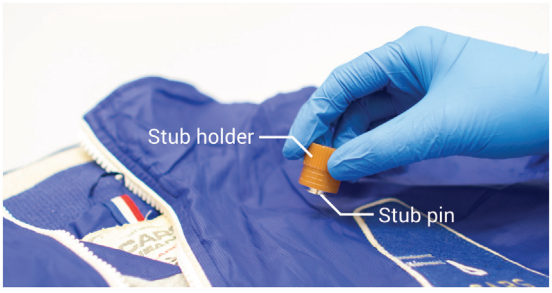

Fig. 2. Tape stubbing is used mainly to collect micro-traces from textiles. This method originates from the Gun Shot Residues collection method, for which a carbon-layer is attached to the stub instead of adhesive tape.

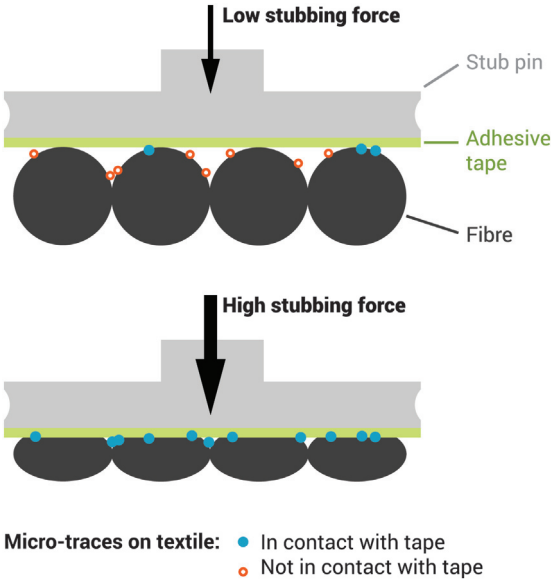

Fig. 3. Schematic representation of the hypothesized effect of the force applied on the stub: (top) with a low force, only a few particles are in contact with the tape. (Bottom) When contact force increases, the contact area between the tape and the fibres increases, enabling collecting more micro-particles.

Therefore, it was hypothesised that stubbing identical samples with a higher stubbing force results in a larger amount of collected micro-traces, as illustrated in Fig. 3. The collection is limited by a maximum contact area and the amount of micro-particles on the substrate. To our knowledge, this is the first time that the effect of contact force on the collection efficiency is studied for trace collection methods in general.

2. Materials and methods

Fluorescent microspheres (Ø25 μm, 12% Coefficient of Variation, Fluoro-MaxTM, Thermo scientific) were used to mock micro-traces that may contain trace DNA. These spheres are sized in the same order of magnitude as skin cells (25 μm) [16], which are one of the components of trace DNA, next to the much larger skin flakes and the much smaller 'free DNA'. Furthermore, these microspheres were visually quantifiable under a microscope.

This study was focused on polyester textile substrates, because polyester is most often present in the textiles sampled in our own case work. Furthermore, because this study was the first of its kind, the textile samples were kept relatively simple to test this new method. To limit the variation between samples and to exclude the effect of textile weavings and compositions, it was chosen to use samples of parallel oriented 100% polyester threads. The experiments were conducted on flat steel spools wrapped in one of three different types of threads: sewing polyester (SP), Ø0.22 mm; extra strong sewing polyester (ESSP), Ø0.35 mm; and crochet polyester (CP), Ø1.13 mm (Fig. 4).

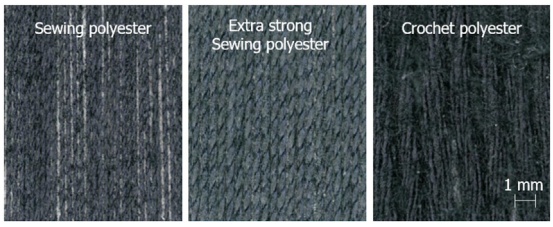

Fig. 4. Microscope images of a sewing polyester ('SP'), extra strong sewing polyester ('ESSP') and crochet polyester ('CP') spool.

The microspheres were deposited on the substrates in a suspension of ethanol. In order to put the microspheres as evenly as possible on the substrates, an as uniform as possible suspension was created by putting it in an ultrasonic bath for 2 min (at room temperature) and frequently shaken thereafter to keep the suspension well-mixed. Next, twenty droplets of the suspension, of 0.025 mL each, with a concentration of 9.2E4 microspheres/mg, were evenly distributed over one side of each spool using a pipette. This amount was chosen arbitrarily to deposit sufficient micro-traces on the samples to allow measuring sampling differences, but avoid excessive coverage of the textile. After drying for at least 12 h at room temperature, the spools were overlain by a masking plate with 5 circular holes to create 5 separate sample locations (Fig. 5).

Aluminium stub-pins were, used as shown in Fig. 6, without stub holders, directly clamped into the setup. Double sided adhesive tape (Scapa 4405, Ø10 mm) was stuck to the surface of the stub-pin (Ø12.7 mm, see Fig. 6). The surface underneath the transparent tape was painted black to improve the visibility of microspheres on the tape.

For forensic purposes, adhesive tape on stubs is UV irradiated before use to make it DNA-free. UV light can degrade polymers and thereby influence the adhesive bonding. Therefore, the effect of the standard UV irradiation procedure on the adhesive bonding was investigated before the tests, which showed no change of the tape's adhesive properties. Consequently, to save time, the tape was not UV-irradiated in this study.

The microspheres were collected from the substrates by stubbing with controlled force. The stubs were fixed onto the head of a tensile tester (Instron, model no.: 4505, serial no.: H2164) with a ±100 N static load cell (Instron, serial no.: 65883), see Fig. 6. The tensile tester was used to control the stubbing force applied during sampling. Five different stubbing forces (maximum normal forces imposed by the tensile tester) were tested: 0.1, 0.2, 0.5, 1 and 7 N. For each combination of stubbing force and substrate material 3 trials were performed. For practical reasons the materials were tested in the order CP, SP, ESSP with the three repetitions per stubbing force grouped per force.

A Keyence VHX-5000 Digital Microscope (Keyence, Osaka, Japan) was used (using image stitching of 200× magnified images) to capture the substrate surface before stubbing and count the number of microspheres placed on the substrate, and to capture the stub-tape surface after stubbing and count the number of microspheres collected on the tape.

The microscope pictures were all identically post-processed in Adobe Photoshop CS6 (Adobe Systems, San Jose, CA, USA) for contrast enhancement in order to discriminate the microspheres from the background. A custom-made MATLAB image recognition script (version R2013b, Mathworks, Natick MA, USA) was used to

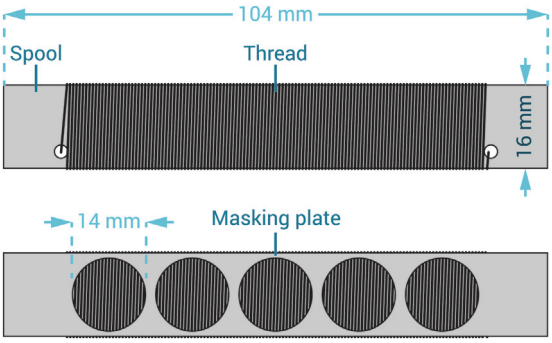

Fig. 5. (Top) Spool wound with thread (one of the types shown in Fig. 4). (Bottom) By covering the thread with an aluminium masking plate, 5 separate, round sample locations (Ø14 mm) were created.

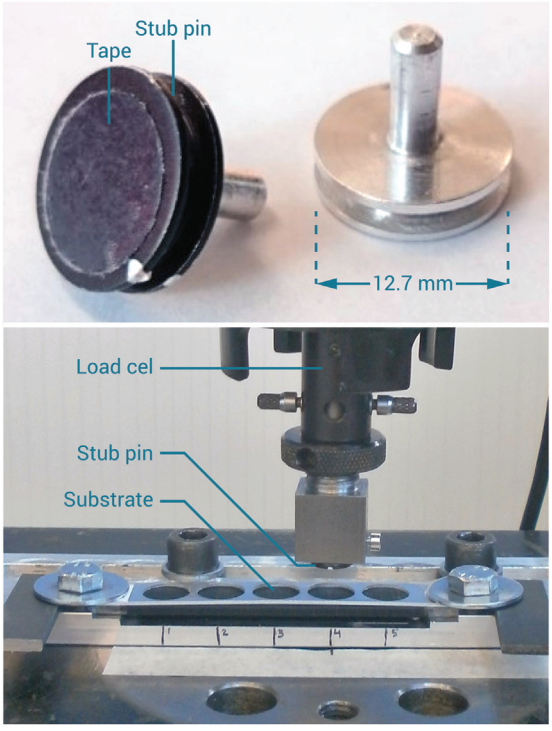

Fig. 6. (Top) Stub pins that were used in this experiment. (Bottom) The spool containing the substrate was clamped to the crosshead of the tensile tester. The stub-pin was clamped to the load cell.

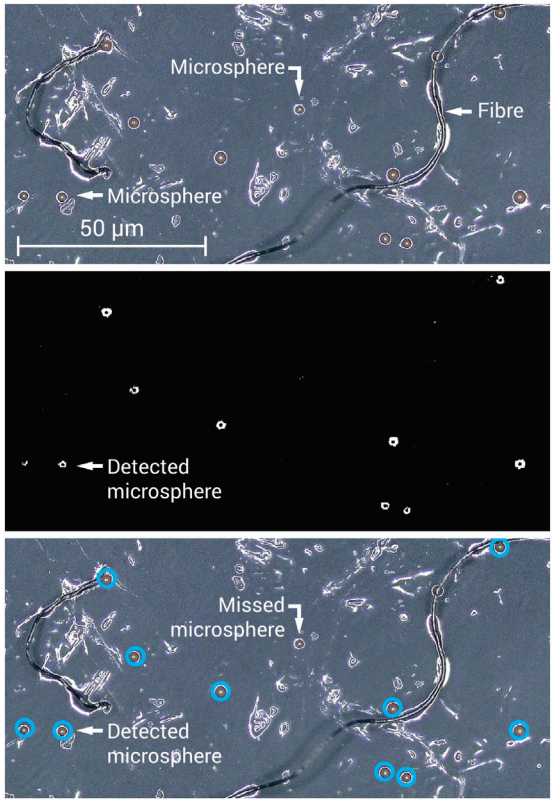

Fig. 7. (Top) Microscope photo of a stub-tape with collected microspheres and fibres on it. (Middle) Image edited in Photoshop. (Bottom) Result of automated particle detection in MATLAB was verified by indicating the location of detected spheres in the original microscope photo, see added thick lined circles.

automatically count the micro-particles in the processed pictures (Fig. 7). All end-results were visually checked for false positive identifications of microspheres, sometimes occurring when, for example, a textile fibre was picked up by the tape and shimmering edges on the fibre were falsely identified as clusters of microspheres. Such clusters of falsely detected microspheres were subtracted from the total number of detected particles.

Part of the end-results, 7 microscope pictures of tape, 2 microscope pictures of both CP and ESSP, and 4 microscope pictures of SP, were visually checked in detail. The absolute number of falsely detected microspheres and the percentage of undetected microspheres were calculated to estimate the method's accuracy.

The collection efficiencies, $\eta_{collection}$, were calculated using Eq. (1).

$$\eta_{collection} = \frac{n_{tape}}{A_{tape} \cdot \rho_{substrate}} \times 100\% \quad (1)$$

Because the exact placement location of the tape on the sample circle was unknown, the number of particles present in the sampled substrate area was calculated by multiplying the particle density on the substrate before stubbing, $\rho_{substrate}$ (average nr. of particles/mm^2), by the surface area of the tape, A_{tape} (mm^2). The number of detected particles on the stub-tape is represented by n_{tape}.

The ambient temperature and relative air humidity near the tensile tester were measured, because changes in these conditions might influence the adhesive capacity of the tape.

3. Results

The test results are displayed in Table 1. In Fig. 8 the resulting collection efficiencies (n = 3 per force level) for the three substrates and five stubbing forces are shown. Curves were fitted through the mean collection efficiencies according to the exponential behaviour $y = -a \cdot e^{-b \cdot x} + c$, with x the stubbing force, y the stubbing efficiency, and a,b, and c the fitting parameters. This trend matched the hypothesis and resulted in a low residual error (Table 2).

The theoretical maximally achievable collection efficiency for each substrate material was taken as the value that its fitted curve approaches asymptotically. The stubbing forces at which the collection efficiency stagnated were defined as the force at which the collection efficiency reached 95% of the theoretical maximum efficiency. The theoretical maxima and the stagnation forces are given in Table 3.

During stubbing, the ambient temperature varied between 21 and 24 °C (mean 22.9 °C). The relative humidity of ambient air varied between 36 and 38% (mean 36.6%, accuracy ±5%). These limited changes in environmental conditions are not expected to have any significant impact on the adhesive bonding of the tape.

Table 1
The number of detected microspheres on the substrate **before** stubbing ('substr') and the number of detected microspheres on the stub tape **after** stubbing ('tape') for each combination of used stubbing force ('F_{stub}') and substrate material ('CP' = crochet polyester, 'SP' = sewing polyester and 'ESSP' = extra strong sewing polyester).

F_{stub} (N)	Trial nr.	Nr. of microspheres detected					
		CP		SP		ESSP	
		substr	tape	substr	tape	substr	tape
0.1	1	549	18	537	8	349	11
	2	695	25	496	15	479	17
	3	630	21	436	1	430	19
0.2	1	797	56	509	15	453	6
	2	717	43	420	11	304	11
	3	830	46	456	9	314	7
0.5	1	671	69	405	28	310	32
	2	573	40	350	34	328	11
	3	737	74	371	14	337	50
1	1	941	146	383	58	302	46
	2	667	125	314	14	329	22
	3	524	104	381	13	323	39
7	1	617	306	272	72	307	70
	2	674	323	348	115	321	73
	3	618	235	354	135	299	68

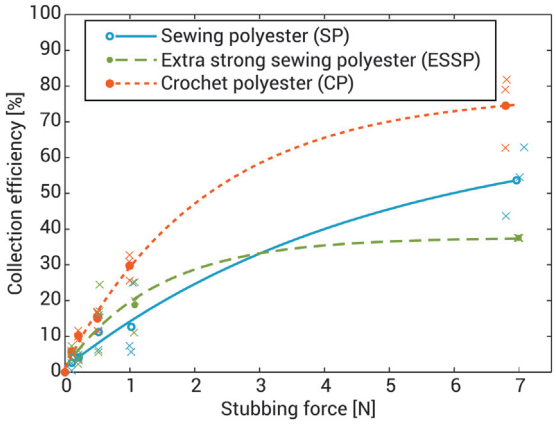

Fig. 8. Mean collection efficiencies (represented by the dots, with each dot being the mean of 3 trials that are each represented by an 'X' sign) for all tested combinations of stubbing force and substrate materials. For each substrate material an exponential curve is fitted through the data points.

Table 2
Parameters of the curves fitted through the collection efficiency (y) and stubbing force (x) relation, and the coefficients of determination for the three tested substrate materials.

Substrate material	Equation	Coefficient of determination (R^2)
SP	$y = -68.12 \cdot e^{-0.21 \cdot x} + 69.32$	0.9934
ESSP	$y = -36.30 \cdot e^{-0.70 \cdot x} + 37.62$	0.9734
CP	$y = -76.63 \cdot e^{-0.45 \cdot x} + 78.00$	0.9972

Table 3
Theoretical maximally achievable trace collection efficiencies, and stubbing forces at which the increase of collection efficiencies stagnated (i.e.: reached 95% of the theoretical maximum efficiency).

Substrate material	Theoretical max. collection efficiency	Stubbing force at stagnation of collection
SP	69%	12N
ESSP	38%	3N
CP	78%	6N

4. Discussion

The results matched the hypothesis that the collection efficiency increases in a similar manner as the actual contact area between the tape and the substrate. The collection efficiency of microspheres increased most quickly among low stubbing forces up to about 1 N. The rise of collection efficiency with increasing stubbing force stagnated between 3 and 12 N, depending on substrate material (Table 3). These results indicate that using a stubbing force higher than 12 N would not be really useful when requiring a maximum collection efficiency on the substrate materials tested. Based on the fitted curves, the theoretical maxima of the collection efficiencies are far below 100%. These results suggest that when aiming at collecting as much trace

material as possible, it might be more useful to stub multiple times with low force on the same location of a substrate than to further increase the stubbing force.

Prior to the conducted measurements, the compressive stiffness of the substrate materials were measured using the same tensile tester as in the tests and a blank stub-pin. This showed that stiffer, and thus less deformable, substrate materials had lower limit collection efficiencies. This might be explained by the fact that more compliant threads allow more compression and deformation and hence enable stubbing deeper into and between the threads. Consequently, it is expected that using more deformable stub pins or thicker, soft stub tapes may compensate partly for the reduced collection efficiency from stiff threads.

4.1. Limitations

The microspheres used in this experiment differed from skin cells in shape and other mechanical properties, which may have affected the adhesive bonding. However, the demonstrated relation between stubbing force and trace collection efficiency is expected to be a valid indication for skin cells, based on the similarity of size (and thus of contact area with the stubbing tape) with these often occurring DNA-containing traces. Additionally, DNA-containing traces do not only exist of skin cells, but also of much larger skin flakes and much smaller free DNA. However, by using the approximately skin-cell sized microspheres the newly developed method for measuring stubbing efficiency could be tested for an intermediate particle size range. Furthermore, using a specific and actual DNA-containing trace would just as well have been a limited representation of the vast range of sizes and material properties of DNA-containing traces, but at a much higher economic cost.

Microspheres might have penetrated in the substrate fibres and then still might have been collected by the tape, despite not having been visible at the surface at the time of analysing the substrate. If so, the actual density of microspheres on the substrate would have been higher than visually quantifiable, so the calculated collection efficiencies would be lower than determined. Additionally, the exact distribution of the microspheres on the substrates was, for practical reasons, not confirmed visually for all samples. However, the suspension holding the particles was constantly kept as homogeneous as possible and applied to the samples in a standardised manner to ensure that all samples could be assumed to have similar microsphere distributions. Random visual checks during pilot tests did suggest that this was the case, but future studies may improve on this by making sample-wide stitched microscope images of each sample.

Because some microspheres were hardly visible and because sometimes glare on shiny edges of textile fibres was detected as a microsphere by the used software, both false positive and false negative counts existed in the data. Manually verifying the outcomes of the detection software in some randomly picked datasets showed that the number of undetected particles was proportional to the number of particles present (about 2–15% depending on substrate material). The number of falsely detected particles (about 28–32 per entire sample) depended only on the substrate material. Therefore the number of falsely detected particles was similar for all samples of the same substrate, regardless of the number of present particles. Without these deviations, the relation between stubbing force and collection efficiency would show to start at a lower collection efficiency and end at a higher collection efficiency, but the general trends and the conclusions drawn from the results would not have differed, as these are only marginal deviations.

4.2. Future research

One could imagine that on rough substrates such as textile, trace DNA can be present on specific depths of the substrate structure only, due to DNA sources that transfer DNA to the substrate during different actions and at different times. To obtain DNA samples that represent a single DNA source instead of a mixture of multiple DNA sources, it would be valuable to get more insight in the distribution of trace DNA along the depth of the substrate structure. In addition, it should be investigated if selectively collecting traces from specific substrate structure depths is possible. Trace collection from only the top layer of the substrate structure could be done when using a very low and controlled stubbing force that barely impresses the substrate. From the relation between stubbing force and trace collection efficiency, it can be deduced that to sample only the top layer, forces in the order of 0.1 N (~10 g) would have to be applied. An unpublished pilot study by Wendt [17] showed that forensic investigators generally use highly varying stubbing forces between 1 and 10 N. Therefore, it is expected that the required forces in the order of 0.1 N cannot be consistently applied manually, but would require an aiding device to control the stubbing force. Such a device has been proposed by Van Eck et al. [18] and enabled controlling the stubbing force between 2.1 and 30 N with a standard deviation of about 0.5 N. Further development of that device to achieve consistent stubbing forces around 0.1 N would be necessary to allow depth-specific stubbing in the future.

As the manual check of the microspheres detection algorithm showed, the algorithm could be improved in terms of specificity and sensitivity. Some improvement could possibly be gained by further improving the manually established image processing that was next automated. However, there are many automated image recognition systems, e.g. for medical image recognition and diagnosis, that outperform humans in terms of speed, specificity and sensitivity thanks to the application of machine learning and deep learning algorithms. Similar developments may greatly enhance the performance of the methods described in the current study.

In order to gain further knowledge about the efficiency of stubbing of different trace materials on other substrates, the current study should be followed up by two types of studies: (1) validation tests investigating whether the results obtained with the used microspheres are transferable to actual skin cells. (2) Tests on other substrates and with trace materials that also mimic skin flakes, free DNA and other materials of interest. This would also show whether the low collection efficiencies and high variations are characteristic for polyester textiles or for the stubbing method in general. Furthermore, these future studies will eventually help establishing quantitative statements about activities related to the transfer of trace-DNA.

5. Conclusion

The presented results strongly support the hypothesis that the collection efficiency of microspheres from textile substrates increases with increasing stubbing force in a concave down increasing function. According to a fit through the measured trace collection efficiencies (measured for stubbing forces of 0.1, 0.2, 0.5, 1 and 7 N), the collection efficiencies seemed to stagnate at 3, 6 and 12 N for extra strong sewing polyester, sewing polyester and crochet polyester, respectively. Stubbing with a force higher than 3–12 N (depending on the substrate material) does not notably influence the collection efficiency. However, because the theoretical maxima of the collection efficiencies were far from 100%, it is expected that stubbing multiple times on the same spot of a substrate increases the total collection efficiency.

Declaration of interests

The authors declare that they have no competing interests.

CRediT authorship contribution statement

Selma Damsteeg-van Berkel: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Fleur Beemster**: Conceptualization, Methodology, Resources, Writing - review & editing. **Jenny Dankelman**: Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision. **Arjo J. Loeve**: Conceptualization, Methodology, Software, Formal analysis, Writing - original draft, Writing - review & editing, Visualization, Supervision.

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