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Forensic investigation of microtraces on an item of Dutch eighteenth century clothing in the Rijksmuseum collection

Yoram Ray Goedhart[1*], Katrien Keune[2,3], Suzan Meijer[2] and Arian van Asten[3,4]

Abstract

Most garments in museum collections have belonged to people of high societal status. Clothing of the common people, however, has rarely been preserved. Because of this, the conservation of such rare items is of special importance. The current study focuses on such an object from the Rijksmuseum collection; a pair of eighteenth century breeches that were retrieved from an anonymous grave on Spitsbergen. As these breeches contain several unidentified materials, it is unclear how best to conduct the conservation of the object. The identification of trace material plays a key role within criminal law and therefore, it is hypothesized that a forensic approach could provide a suitable framework within this case of cultural heritage as well. After forensic examination, trace material was analyzed using a microscopy-based approach and was found to be mainly of biological origin. Energy-dispersive X-ray (EDX) and Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy indicated the presence of human skin remains. It was concluded that a modern forensic approach can successfully be applied to a historical object within cultural heritage and can assist decision-making regarding possible future conservation of the object. The systematic application of the forensic methodology was found to be appropriate with little to no modification to classify and identify trace materials, but further improvements could be made. The current work should be seen as a starting point that allows for more questions regarding museum objects to be answered in a forensic manner, including aspects such as authenticity, the chain of custody, the context, original use and object conservation.

Keywords: Microscopy, SEM–EDX, ATR-FTIR, Cultural Heritage, Forensic Investigation, Human Biological Traces

Introduction

Most historic garments in museum collections have belonged to people of noble or royal status because these valuable items are well taken care of and conserved and protected over time. Clothing of the common people, however, is rarely preserved limiting the contemporary understanding of everyday living conditions in the past. At the time, these garments were not perceived as valuable enough to save and collect and, as textiles were scarce, were mended until they could no longer be worn before being cut up to serve a different purpose. Therefore, as these items of clothing are uncommon in museum collections, their conservation is of the utmost importance.

The current study focuses on such an object: a pair of Dutch eighteenth century fly-front breeches from the Rijksmuseum collection (Fig. 1). The breeches (dated ca. 1750 [1]) originate from the grave of a Dutch whaler on the small island Zeeuwse Uitkijk, Spitsbergen (Norwegian: Ytre Norskøya, Svalbard). According to the anthropological analysis, the object is thought to have been worn by a man who died around the age of twenty-two years old by an unknown cause [2]. An important aspect regarding the origin of the object is that the breeches have not been cleaned upon retrieval, in contrast to other textiles of the Spitsbergen collection [1].

*Correspondence: y.r.goedhart@gmail.com

[1] Netherlands Forensic Institute, P.O. Box 24044, 2490 AA The Hague, The Netherlands
Full list of author information is available at the end of the article

Fig. 1 Woolen fly-front breeches (NG-2006-110-16) retrieved from a grave on Zeeuwse Uitkijk, Spitsbergen. The item has not been cleaned and contains highly fragmented material, possibly including human remains. Photo: Rijksmuseum collection, Amsterdam

The breeches still contain materials that were previously assumed to be from the grave site. This, mostly unattached, material is highly fragmented and unidentified. The unattached material on the breeches will be referred to as 'traces' or 'trace material'. The trace material poses a challenge for the object's conservation. As the composition of the trace material is unknown, it has remained unclear whether the trace material holds any value and therefore, what the rightful approach is for conservation of the object.

This study aims to aid the conservation process by subjecting the breeches and the trace material to a forensic investigation. The identification of various traces and their origin play a key role in criminal investigations. As these questions are also relevant in this case, it is hypothesized that a forensic approach could also provide a strong framework for the objective collection of information within a museum context. The potential of forensic science within cultural heritage has already been demonstrated through analytical techniques that are shared between the two fields, for example regarding the detection of art forgeries or material identification [3–6]. These studies mainly seem to focus on materials such as paint, binders, metals and textile dyes [7–9] to investigate the materials and methods used by the artist. In this case study, the questions revolve around the composition and nature of the traces and whether they could be authentic. In addition, the highly structured approach and evidence-based argumentation of a forensic investigation could yield information that can subsequently be used to either support or refute the assumptions that are presented in the existing documentation.

In the museum dossier it is mentioned that the trace material contains remnants of pubic hair and skin [1]. Interestingly, remains of human nails have also been found on other items of clothing from this collection. The possibility that biological remains could indeed have been preserved is not entirely unlikely. Under favorable conditions, hair, nail and skin are known to remain intact over a long period of time [10]. A cold environment that promotes desiccation and inhibits microbial activity, such as Spitsbergen, is usually most favorable for the survival of these keratinous matrices [11]. The soil of Spitsbergen is characterized by permafrost and contains no animal life. The low temperatures and the acidic condition of the soil further contribute to a favorable environment for material preservation [1, 10]. This is also supported by studies that examined ancient hair recovered from permafrost [12, 13], or the findings of ice-mummies [14].

By identifying the trace material and examining the breeches, relevant questions regarding origin and state could be answered. This information is relevant to obtain a better understanding of the context of the object and to assist in decision making for conservation, *e.g.* whether removal of the trace material from the breeches could be favorable for preservation. In addition, trace findings could possibly also serve as a source of interesting information for museum visitors and could therefore also be part of the display of cultural heritage objects. However, for this approach to be successful, it needs to be assessed whether forensic (micro) trace analysis can be applied to objects that are not several weeks or months but rather several centuries old.

A brief overview of Dutch whaling

At the end of the sixteenth century, the growing Dutch population led to an increasing demand for oils that were required for lubricants and fuel. Combined with a shift in agriculture the supply of plant-based oils was no longer sufficient. Therefore, the production of animal-based oils became of greater interest [15]. During the same period, the arctic waters were explored by Dutch seafarer Willem Barentsz, who became most famous for his voyage to the island of Novaya Zemlya (Nova Zembla) and the discovery of the Svalbard archipelago (also known as, and hereafter referred to as, Spitsbergen). The observations made during those journeys combined with the need for oils eventually led to the emergence of Dutch whale hunting around 1620 [16]. The Dutch whalers built settlements in the north-west region of Spitsbergen, the largest one being Smeerenburg on Amsterdam Island [16]. From mid spring to the end of summer, the Dutch ships sailed annually to the arctic [17]. During this period, the whalers lived and worked in the settlements. Whales were killed, dragged ashore and their blubber was cooked to oil. However, it didn't take long for the settlements to become abandoned. Around 1670, the arctic summer period became shorter due to climatological conditions and the whales remained at open sea due to permanent ice formation in the bays [15, 18]. Consequently, the whalers had to sail further distances and could no longer bring the whales ashore and both the hunting and chopping of whale blubber had to be done at sea. The oil was then cooked upon return in the Netherlands. This shift meant that the work of the whalers became even more dangerous, and many men did not survive the harsh conditions. With the settlements abandoned, the whalers only came ashore to bury their dead. Dutch whaling eventually came to an end around 1800 [15].

The Smeerenburg project

In 1980, the remains of the settlement and the cemeteries at Smeerenburg and on the small island Zeeuwse Uitkijk were investigated during the so-called Smeerenburg project by the Arctic Centre of the University of Groningen. Human remains of fifty out of 184 graves on Zeeuwse Uitkijk were exhumed. Surprisingly, the graves contained many remains of textile in good condition, among which a few complete pieces of clothing, such as the breeches (Fig. 1).

The 1980 Smeerenburg project did not have a forensic focus and the approach during that time differed from contemporary insights. Therefore, the retrieval of the object was not in agreement with modern forensic guidelines on evidence handling. These guidelines are of importance to ensure that traces are not transferred between different pieces of evidence. The textiles on Spitsbergen, however, were retrieved from the graves without gloves or other protective equipment that would otherwise prevent contamination. Moreover, pieces of clothing were rolled up in old newspapers before further packaging (Archaeologist M. Hoogland, personal communication, April 3, 2020). As a result, contamination from the archaeologists themselves or other graves cannot be ruled out and should be kept in mind during the examination.

For transport, the textiles were sealed within a plastic bag filled with water to avoid dehydration. However, the packaging of the breeches started leaking and the textile completely dried up, luckily without the development of a fungal infestation. Upon arrival in the Netherlands, all textiles except for the breeches, were rinsed with demineralized water and in some cases treated with a formaldehyde solution to prevent fungal growth [1]. The breeches including the trace material were kept in the condition in which they arrived in The Netherlands.

Once in the Netherlands, the whereabouts and condition of the object becomes unclear. Documentation regarding the breeches is scarce and likely incomplete (Textile archaeologist Dr. S. Comis, personal communication, April 1, 2020). Besides a Rijksmuseum exhibit in 1988, there is no other documentation suggesting that the breeches have been on additional display, although this cannot be fully ruled out. While it is assumed that the breeches mainly have been kept within institutions with restricted access, a clear chain of custody cannot be reported. This refers to the chronological documentation of evidence transfer and ensures safe-keeping of trace material. According to Dr. S. Comis, the textile archaeologist who worked on the Spitsbergen collection since its retrieval in 1980, and the Dutch Cultural Heritage Agency, which also kept part of the Spitsbergen collection, the items within the Spitsbergen collection have been stored in multiple places as it was unclear at that time where the collection should go due to financial reasons (personal communication, April 1, 2020). Therefore, the items quickly got separated after retrieval. Since the exhibition in 1988 in the Rijksmuseum it is assumed that the breeches are in collection of the Rijksmuseum. It is unclear where the breeches were kept between 1980 and 1988. Within a forensic investigation, this lack of a clear chain of custody would raise questions regarding the integrity of the evidence, or in this case, the information available regarding the breeches and the trace material. This also points out the relevance for the Rijksmuseum to conduct a forensic examination of this object in the current study.

Since the original investigation aimed at the technical analysis of the textiles and description of the garments, information regarding the trace material is not present in the available documentation except for a brief note in the dissertation of textile archaeologist Comis [1]. Within the official documentation, only the museum photos provide some indication. It shows that while some trace material is adhered to the textile, other material appears to be loose and in shifting position in the various pictures taken. This suggests that the location of the mobile traces will probably not be of much relevance.

Case study approach

The current work will explore to what extent a modern forensic approach can be used to objectively gain relevant information regarding an object within cultural heritage. To this end, the object will be treated as physical evidence subjected to a forensic trace examination.

This examination will be conducted according to existing and currently used protocols [19–21],[1] and is supported by formal documentation and forensic photography as described in literature and documents used by the Netherlands Forensic Institute (NFI) [22–26].[1] X-ray fluorescence (XRF) will be used to study the breeches and the trace material before these are manipulated. Samples of the trace material will be taken for further investigation using both destructive and non-destructive methods, with a preference for the latter to conserve as much of the trace material as possible. As various disciplines in forensic science rely on human observation-based expertise as the main method of identifying microtraces, the collected traces will be visually analysed using microscopy with magnifications ranging from 10 to 500×. The observer-based identification of trace materials includes expert consultation as this requires a high level of expertise and experience. To obtain detailed chemical information, a small selection of the trace material will be analysed using spectroscopic methods. The structure, surface morphology and elementary composition of the sampled material will be studied using Scanning Electron Microscopy with Energy-Dispersive X-ray spectroscopy (SEM–EDX). For more specific chemical characteristics, Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) will be used. The aim is to combine the results from the various investigations to classify and possibly identify the trace material to enable informed decisions with respect to the conservation process.

Methods

Documentation

Formal documentation forms have been created based on those used by the Netherlands Forensic Institute (NFI) and descriptions from literature. Forms were adapted to the current study, but not specifically designed for cultural heritage cases only. A forensic clothing examination form, a trace collection form and a general observation and examination form were created (See Additional file 1: Figs. S1–S6 for empty forms).

Visual examination

The examination took place in the Rijksmuseum textile conservation studio according to the existing protocols. The breeches were examined first with the naked eye under natural and artificial white lighting, as well as with a common UV lamp. The outside of the breeches was examined first, followed by the inside where accessible. To avoid movement and possible loss of loose trace material, the breeches were not turned over. The backside was examined separately after trace collection. To this end the breeches were transferred to a glass plate that was placed in a cardboard frame.

Sampling

With the use of tweezers, samples were mostly taken from the inside of the breeches, as it is assumed that human material, if present, is more likely to be found there. The inside is also expected to have been less exposed to sources of contamination. Samples were packaged in glassine envelopes according to the beforementioned protocols. Large fragments (>5 cm) were collected in plastic jars. Traces that were relatively fixed in place or of notable size were taken separately. A bulk sample of the highly fragmented and commingled traces was taken from both the inside and the outside of the breeches. Only a small amount of this loose material was taken to obtain a representative sample. Hairs and fibers were collected on hair collection cards created by the NFI, one card for traces on the outside and another card for traces on the inside of the breeches.

Microscopy

First, the trace material was examined using a Leica MZ7.5 Stereomicroscope (Leica microsystems, Wetzlar, Germany). Subsequently, the traces were analysed with a Hirox KH-8700 3D Digital Microscope combined with the MXB-2500REZ revolver lens (Hirox Europe, Limonest, France). For a more detailed analysis, samples were placed on a glass slide and examined using the Zeiss Axio Imager 2 Pol with Axiocam 506 color and the Colibri.2 light source for UV lighting (Carl Zeiss Microscopy GmbH, Gottingem, Germany). Images were captured using the corresponding Zeiss ZEN Pro software (version 3.1).

Smear slides were made for samples which suggested to contain material with cell structures, using Meltmount 1.662 (Cargille Laboratories, Ceder Grove, NJ, USA). These slides and the collected hairs/fibers were examined using the Zeiss Axio Lab.A1 transmitted light microscope (Carl Zeiss Microscopy GmbH, Gottingem, Germany).

XRF

The Olympus Delta Professional Handheld XRF Analyzer with Rh tube (Model DPO-2000-CC, Olympus IMS, Waltham, USA) was used as an indicative tool to determine the elemental compositions of various areas (1 × 1 cm). Measurements were made at 10 and 40 kV for 8 s (Geochem mode). The measured areas are given in Additional file 1: Fig. S7. Based on the initial results, it

[1] Sources 19–23 derived from the intranet (not publicly accessible) of the Netherlands Forensic Institute. These documents are part of the quality system of the NFI, please contact the NFI for more information (https://www.forensicinstitute.nl/contact).

Fig. 2 Overview of the breeches. The breeches as seen during the forensic examination with closed (**A**) and open (**B**) fly-front. Inside, a dark grey material can be seen that is adhered to the fabric and represents most of the trace material

was decided not to continue with a macro-XRF scanner (MA-XRF).

SEM–EDX

The FEI Nova NanoSEM 450 (Thermo Fisher Scientific, Waltham, MA, USA) was used, operating at low vacuum (90 Pa) and 20 kV accelerating voltage, with LVD and EDX detectors for secondary electron imaging and EDX spectra, respectively. Small subsamples of the selected trace material were taken using a forceps and placed on aluminium stubs using copper tape. EDX analysis was supported using Pathfinder software (Thermo Fisher Scientific, Waltham, MA, USA).

ATR-FTIR

ATR-FTIR on the same subsample fragments taken for SEM–EDX was performed using a Perkin Elmer Spectrum 1000 FTIR instrument combined with a GrasebySpecac Golden Gate Single Reflection Diamond ATR, from 4000 to 400 cm^{-1} for 16 scans at a resolution of 4 cm^{-1}.

Expert consult

A forensic biologist, an archaeobotanist, two forensic textile and fiber experts and a forensic archaeologist were contacted to assist with the trace material classification and, if possible, identification. The experts were provided with photographic material and limited context information as to avoid possible bias.

Overview samples and applied techniques

All samples were examined by microscopy. A small selection has been analysed using SEM–EDX and ATR-FTIR. An overview of the applied methods per sample can be seen in Additional file 1: Table S1.

Results

Forensic trace examination

Newly created documentation forms were used and supported by photography (Additional file 1: Figs. S8–S13). While the inside of the upper half of the breeches remained quite accessible, the leg parts were not, as the textile had become quite stiff. The inside, and parts of the outside, contain a black/grey material that is adhered to the fabric and constitutes most of the trace material (Fig. 2). The surface texture of this material shows a craquelure pattern (close-up available in Additional file 1: Fig. S14). Based on this observation, it is assumed that the fragments of this material originate from a single source.

Examination with a UV lamp was conducted in complete darkness. No stains (either visible or latent) or specific fragments showed any fluorescence. Only small fibers, assumed to be dust or particles from modern

Fig. 3 Appearance of the two most abundant materials recovered from the breeches. **A** An amorphous material, that appears to coat most of the inner breeches (50×, Dark Field). **B** Textile remains of natural fibers. The textile is very dirty and combined with other materials. Shown are some fiber bundles that are relatively clean and separated from the woven structure. As can be seen, the bundles are flat and with little to no twist (50×, Dark Field)

packaging, were fluorescent and mostly present on the outside of the garment as opposed to the inside.

Using a supporting glass plate, the backside of the breeches was examined and inspected for the very first time. The backside appeared relatively clean compared to the front and contained fragments and discoloration patches that were assumed to originate from wood from the bottom of the coffin or wood shavings, which were a known filling material of these graves [1]. Two woolen repair patches were found, as well as a large tear along the left pant leg. The threads of the repair patches were degraded, indicating that these likely consisted of cellulose fibers (e.g. linen or hemp fibers).

Trace collection

A total of 7 samples was taken from the loose material surrounding and inside the breeches and documented using the trace collection form (Additional file 1: Figs. S12, S13). Bulk samples four and six were later subdivided into 6 and 3 new samples, respectively, based on the observations by stereomicroscopy. In addition, 18 hairs and fibers were recovered, 9 on the inside and 9 on the outside of the breeches leading to a total of 32 samples.

Microscopy

Using stereomicroscopy, a general inspection of all samples was conducted. It appeared that the sampled material was mostly brown, very brittle and that clear structures were often lost (for a visual overview of the sampled materials, see Additional file 1: Figs. S15-S17). Yet, it could be determined that the collected traces predominantly consisted of organic material.

The samples were further investigated using the Hirox digital microscope and Zeiss Axio Imager research microscope using multiple exposure settings. Based on visual appearance most of the traces could be grouped in two classes: an unknown black/brown flaky material with no clear structure and textile remains made from flat natural, likely bast, fiber(bundle)s (Fig. 3).

Regarding the retrieved hairs, it became clear that while some of them were severely damaged, others were in good condition. This could suggest that some trace material is from more recent origin and linked to contamination. Moreover, whereas most human hairs found appeared to be cylindrical, others appeared to have a more oval shape, indicating that these specimens do not originate from the scalp. Finally, a few textile fibers were found as well as two animal hairs. These hairs are different in color, show spinous scales, frayed fibrils at the roots and a uniserial medullar ladder.

Table 1 provides an overview of the traces retrieved and includes associated descriptions. As can be seen, several samples are visually very similar, whereas others appear to have more specific characteristics.

The Hirox digital microscope also allowed the examination of trace material that remained attached to the breeches. Due to the textile-like material found in the samples, this microscope was used to characterize the textile traces on/in the breeches. It appeared that four types of textile fibers were present: degraded textile remains like those found in the samples, wool that appears to be the same as that of the breeches itself, a

Table 1 Overview of the main characteristics per sample as assessed by light microscopy

Sample ID	Main characteristics
1	Brown, flaky, transparent
2	Black, yellow, fibrous
3	Brown, flaky, textile-Like
4.1	Brown, flaky, transparent
4.2	Brown, transparent, speckled
4.3	Fibrous
4.4	Black, irregular
4.5	Brown, cylindrical, heterogenous
4.6	White, transparent, veins, scales, wing-like
5	Brown, flaky, transparent
6.1	Brown, flaky, transparent
6.2	Textile-Like
6.3	Brown, wood-like
7	Black/brown, flaky, transparent
8.1	Hair root, cylindrical, clean
8.2	Hair root, cylindrical, clean
8.3	Twisted fibers, shiny, transparent, homogenous
8.4	Hair root, continuous medulla, oval, dirty
8.5	Fibrous, wood-like
8.6	Fibrous, flat, transparent, heterogenous
8.7	Hair root, brown, spinous scales, uniserial ladder, clean
8.8	Hair root, continuous medulla, oval, dirty
8.9	Twisted fibers, shiny, transparent, homogenous
9.1	Hair root, white, spinous scales, uniserial ladder, clean
9.2	Fibrous, frizzy, irregular
9.3	(Hair) continuous medulla, oval, dirty
9.4	Hair root, cylindrical, clean
9.5	Hair root, cylindrical, dirty
9.6	Hair root, cylindrical, dirty
9.7	Hair root, cylindrical, clean
9.8	Hair root, fragmented medulla, cylindrical, dirty
9.9	Hair root, fragmented medulla, cylindrical, dirty

Sample IDs and their main visual characteristics are described

polyester thread and a textile fragment of natural fibers (Fig. 4). The polyester thread was found in a zig-zag stitching pattern. This was later confirmed as a means to keep the underlying trace material in place (Textile archaeologist Dr. S. Comis, personal communication, April 12, 2021). The use of a synthetic fiber material for item repair and fixation was deliberate to clearly indicate its exogenous nature. The textile fragment seems to be of a natural origin as used in the seventeenth and eighteenth century, like hemp or linen, but also seems to be in good condition and no other fragments like this have been found. Therefore, it could also be possible that this trace is not authentic but the result of contamination.

Transmitted light microscopy

Smear slides were made using samples 1, 2, 3 and 6.1 as these appeared to be different materials, yet remained hard to characterize using a greater magnification beyond the range of the stereomicroscope (10–50×). It was expected that sample 3 contained a combination of textile remains and the same material as sample 6.1. With this type of microscopy, the trace materials remained to appear amorphous and no new information such as (cell) structures or other characteristics was obtained (see Additional file 1: Fig. S18). The material of sample 2 was found to be unsuitable for this method as it did not transmit any light, differentiating itself from the other samples and possibly made up of compacted soil. While hair and fibers were examined as well, the use of transmitted light microscope again did not yield any useful additional information for these traces.

Handheld XRF

Using the handheld XRF a combination of Si, Fe and Ca was found for all areas measured, an elemental combination which is often associated with soil. However, no distinctive elements or profiles were obtained for specific materials and locations (for most common elements, see Additional file 1: Table S2). Therefore, it was decided not to continue with spatial resolved XRF mapping using a macro-XRF scanner because of the limited differentiation potential of the trace material with this technique. For more detailed elemental impurity profiling ICP-MS would have to be used but this is a destructive technique that requires a considerable sample size.

SEM–EDX

Four samples were selected for further investigation, based on their visual characteristics; sample 6.1 (which represents most of the material covering a big part of the inner part of the breeches, thus being the best candidate for human material); sample 7 (fragments that have similarities to those of 6.1, but appear to be less irregular raising the question whether these two samples contain the same material); sample 5 (a large fragment that is assumed to be of botanical origin); and sample 4.2 (a thin brown material that provides no visual clues whether it could be of botanical or human origin and for which SEM–EDX could yield new insights).

The secondary electron images revealed compact structures (Fig. 5). However, no micro/fine structures were seen that would allow a more specific physical characterization. Some crystalline structures were visible in the secondary electron images of sample 7, which EDX pointed out to be carbon-rich. The overall structure of this sample was comparable to that of

Fig. 4 Different textiles found on the breeches. **A** a polyester thread (Tetex, 140× magnification), **B** woven textile in a degraded state made of natural, likely bast, fibers (35× magnification), **C** a piece of woven textile, different from **B** with a higher density and more twist (35× magnification), **D** wool, which makes up the breeches themselves and the string attached to it (35× magnification)

sample 6.1 and appears to consist of flat, thin layers. The surfaces of sample 4.2 and 5, however, appear to be more uneven compared to the flat flakiness of samples 6.1 and 7.

Below, typical EDX spectra for the four samples are depicted (Fig. 6). The backscatter electrons indicated a very low level of elemental contrast. Additionally, for samples 6.1 and 7 the spectra clearly indicate the presence of C, N and O, as well as Ca, S, P and a trace amount of Mg. The substantial signals for C and O indicate that the traces are predominantly of an organic nature. The presence of nitrogen could indicate a peptide bond, thus the presence of proteinaceous material. The other two samples, 5 and 4.2, also include elements such as Si, Al, Na and K. The combination of Si, Al and Na is indicative for soil. Given the environment from which the trace material was taken, a possible compound from which P and Ca could originate is bone mineral which consists of carbonated hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). Solely based on the surface morphologies and differences in the EDX spectra, there seem to be two types of material present. However, this hypothesis was not supported by subsequent ATR-FTIR analysis.

ATR-FTIR

The selected samples 6.1, 7, 5 and 4.2, were subsequently analyzed using ATR-FTIR. The four spectra appear to be very similar (Fig. 7). As mentioned above, this group of subsamples was selected based on the expectation that they would represent two material classes. Thus, these spectra suggest that all traces have the same organic chemical composition but have taken on different physical morphologies due to different conditions over many years at the grave site.

Around 3290 cm^{-1} a broad band is found corresponding to intermolecular hydrogen bonds indicative for the presence of OH groups often in the form of water. The peaks at approximately 2920 and 2850 cm^{-1} likely derive from CH_2 and CH_3 stretching, which is indicative of lipids [27]. Sample 7 also shows more sharp peaks here. The bands found at 1630 and 1538 cm^{-1} are ascribed to amide functionality (associated with C=O stretching and N–H bending and C–N stretching, respectively). These findings are of special interest, indicating the presence of protein. The band near 1030 cm^{-1} (stretching of C–O in a hydroxy group) is most likely not part of the protein itself, but from minerals. As stated earlier, a possible mineral that is present in the samples is the bone mineral

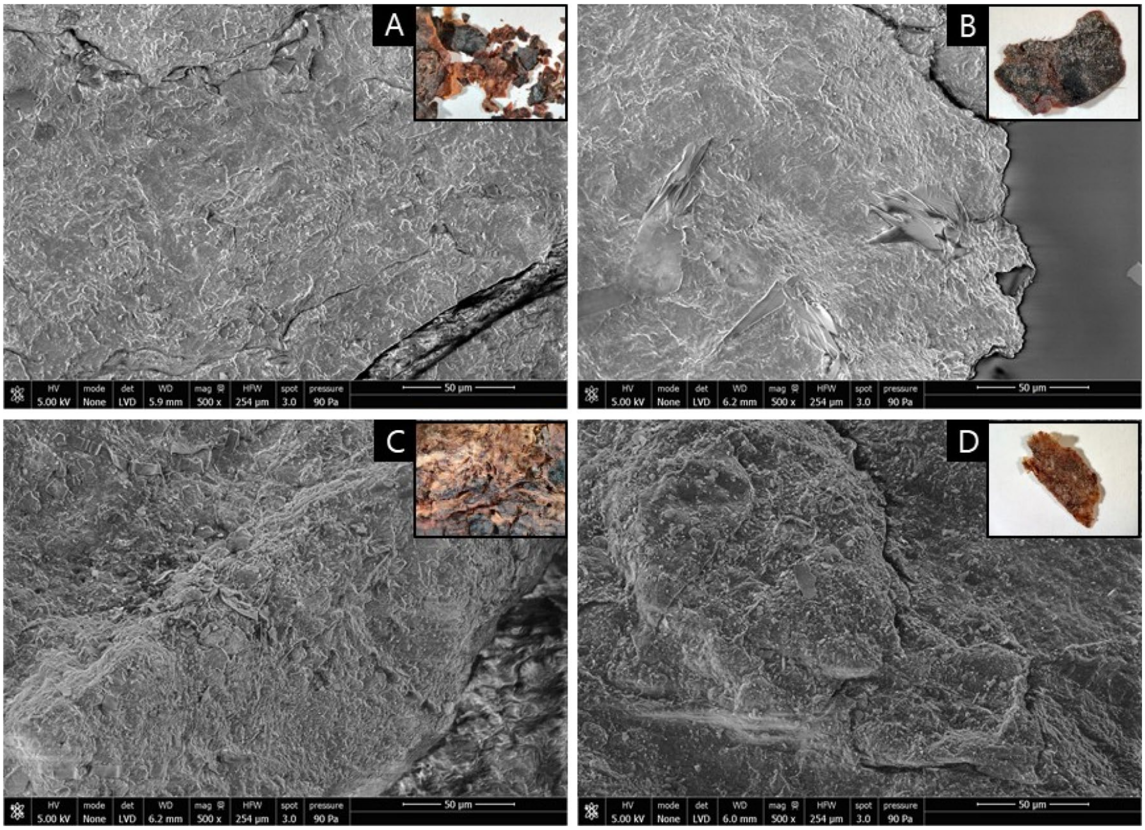

Fig. 5 Secondary Electron Images of the selected trace material at 500× magnification. Shown in the upper right corner are the traces under 10× magnification, which were used to obtain the SEM images. **A** (sample 6.1) and **B** (sample 7) show similar structures. Some crystal structures are also visible for **B**. **C** (sample 5) and **D** (sample 4.2) showed similarities for these samples but with significant differences compared to **A** or **B**

hydroxyapatite, due to the presence of P and Ca as indicated by the EDX spectra. However, the 1030 cm^{-1} band could also simply derive from soil minerals as elements Si, Al and Na were also determined with EDX.

Expert consult

All experts that were consulted pointed out the difficulty they experienced classifying the material, due to extent of sample degradation and soiling. Nonetheless, expert consult enabled the identification of some traces and confirmed earlier classification steps.

Fibers and textile-like materials of samples 3, 6.2 and 8.6 were identified as natural bast fibers, with 8.6 being specified as linen. The textile remains left on the breeches that were examined with the Hirox digital microscope were also classified as bast fibers. Samples 6.3 and 8.5 were identified as botanical remains, namely wood and part of a plant stem, respectively. Finally, the fragments of sample 4.6 were identified as insect wings that may originate from the Lepidoptera family (including insects such as butterflies and moths), based on the presence of scales.

Classification

Based on the observations made with microscopy and the results from SEM–EDX and ATR-FTIR, the samples have been sorted into different classes to elucidate the composition of the trace material. Most of the traces share characteristics and form clusters (Table 2). A diagram to classify these clusters is provided below (Fig. 9). Only nine out of 32 samples are not part of a cluster and form a single class based on their characteristics.

The samples in clusters 1–3 were identified as human material. The first cluster represents a majority of the trace material and is characterized by its brown flaky appearance. This cluster also includes the trace material subjected to SEM–EDX and ATR-FTIR. The EDX results already indicated that these samples consisted mostly of organic matter. The presence of the amide I and II bands found with ATR-FTIR indicate that these materials are most likely not botanical. Combined with the flaky and semi-transparent morphology seen during the microscopic examination and the additional indication of lipid presence using ATR-FTIR (lipids being an important element of the skin structure), it is believed that the

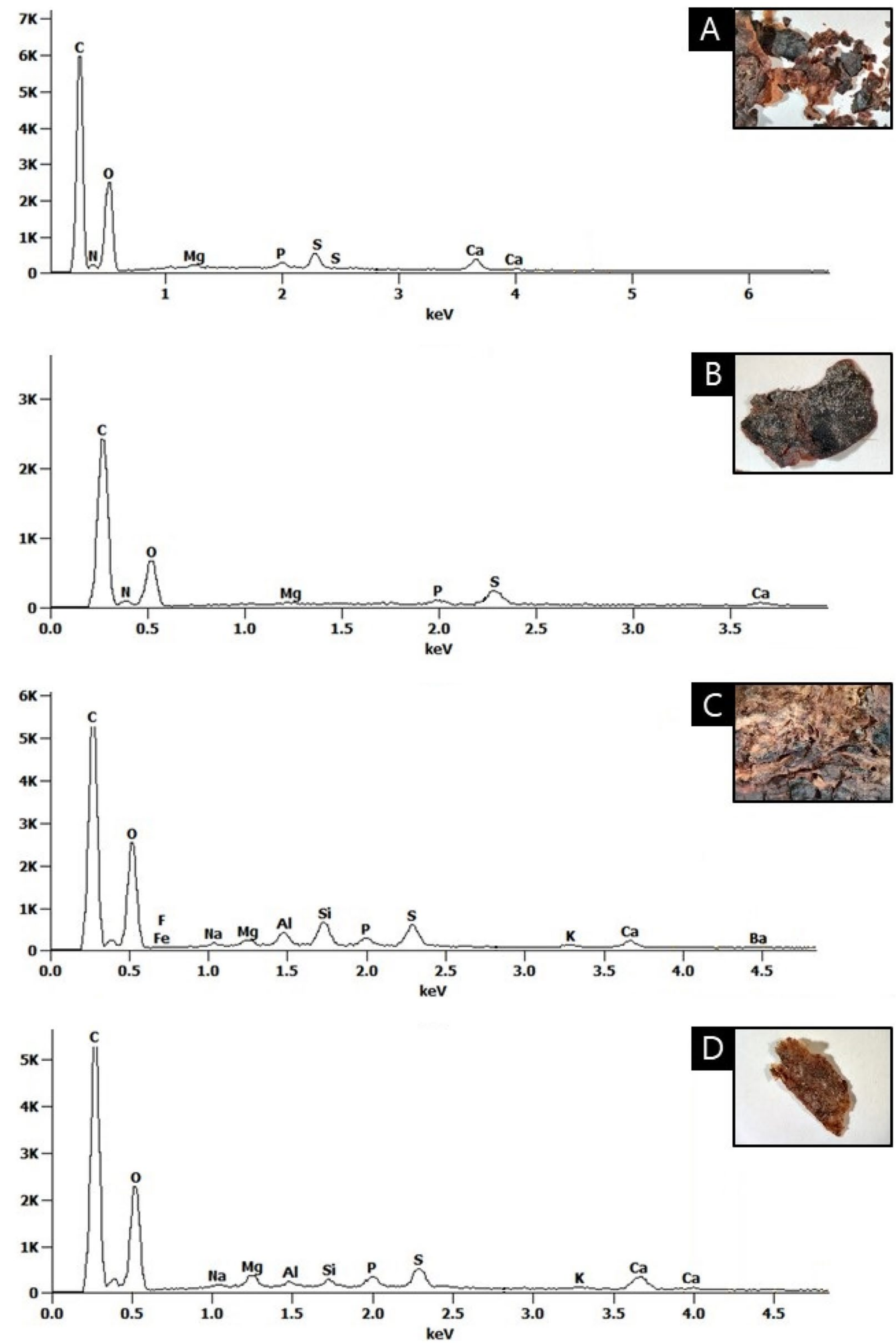

Fig. 6 Energy Dispersive X-ray spectra of the selected trace material

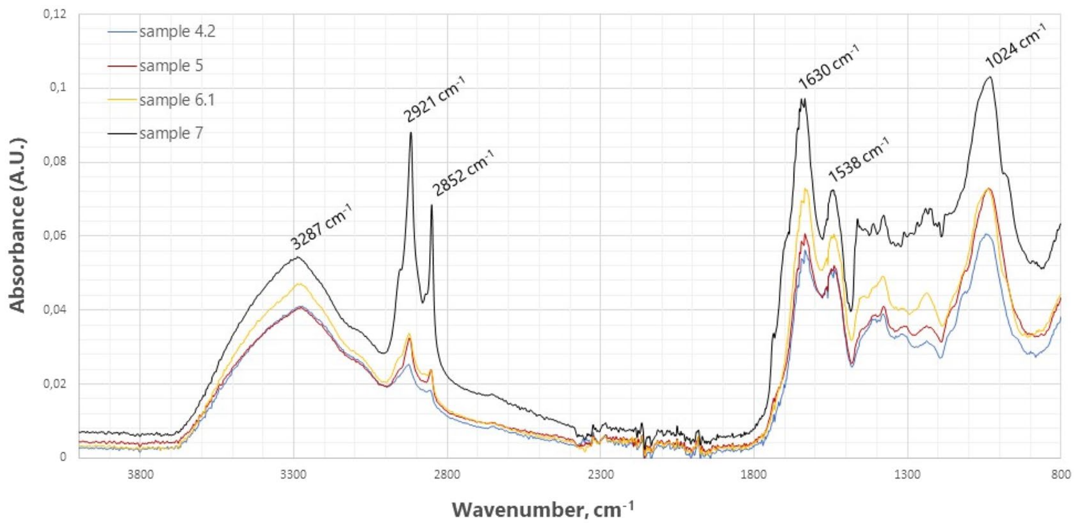

Fig. 7 ATR-FTIR spectra of the selected trace materials

Table 2 Overview of trace clustering (in total 6 classes containing 2 to 8 traces)

Cluster	Sample IDs	Commonalities	Believed origin
1	1, 3, 4.1, 4.2, 5, 6.1, 6.3, 7	Brown, flaky, transparent	Organic
2	8.1, 8.2, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9	Hair root, cylindrical	Organic—Human
3	8.4, 8.8, 9.3	Hair root, continuous medulla, oval	Organic—Human
4	8.7, 9.1	Frayed hair root, spinous scales, uniserial ladder	Organic—Animal
5	8.3, 8.9	Fibrous, shiny, transparent, homogenous	Inorganic
6	3, 6.2	Textile-like	Organic—Plant
Remaining: 2, 4.3, 4.4, 4.5, 4.6, 6.3, 8.5, 8.6, 9.2			Various origin

analyzed materials are the remains of dried skin. It is also this combination of lipids and protein that is characteristic for skin as opposed to other proteinaceous material. However, other human putrefaction residues cannot be ruled out as potential contributor to this trace material.

Cluster 2 and 3 represent human hair. Based on shape, it is concluded that cluster 2 contains scalp hair, whereas cluster 3 relates to body hair. It appears that a part of the scalp hair (4 out of 8, note that these were mostly sampled on the outside of the breeches) are in pristine conditions with no damage or dirt present. This could indicate that these hairs are not associated with the original wearer of the breeches (or individuals involved in the burial) but originate from the investigators or museum personnel handling the object. In line with this proposition the other scalp hair fibers and body hairs are very soiled. These hairs seem to be coated in another material that could be distinguished from the hair using UV light at 365 nm (Fig. 8). Indication of fungal infestation was also visible within these hairs, which presented itself as fungal tunneling (i.e., the process in which keratinolytic fungal spores bore through the hair cuticle and digest the cortex from within). A further subdivision to estimate the number of hair donors was not carried out, as microscopic hair examination is known to lack sufficient scientific basis for donor identification and forensic comparison [28]. However, nine out of the eleven human hairs found contained the hair root, indicating that genomic DNA might be present and could therefore be subjected to further analysis.

Cluster 4 consists of the two animal hairs. The frayed fibrils at the root, uniserial medullar ladder and spinous scales indicate a feline origin [29]. These strands are most likely the result of contamination as dogs and chickens were the only animals accompanying the Dutch whalers [30].

The final two clusters are identified as textiles. Cluster 5 is identified as polyester fibers, whereas cluster 6 contains remains of a natural bast fibers in a woven form. This group of fibers includes flax (from which linen is made), jute, hemp and nettle. As these fibers originate from plants and thus consist of cellulose, they are quickly

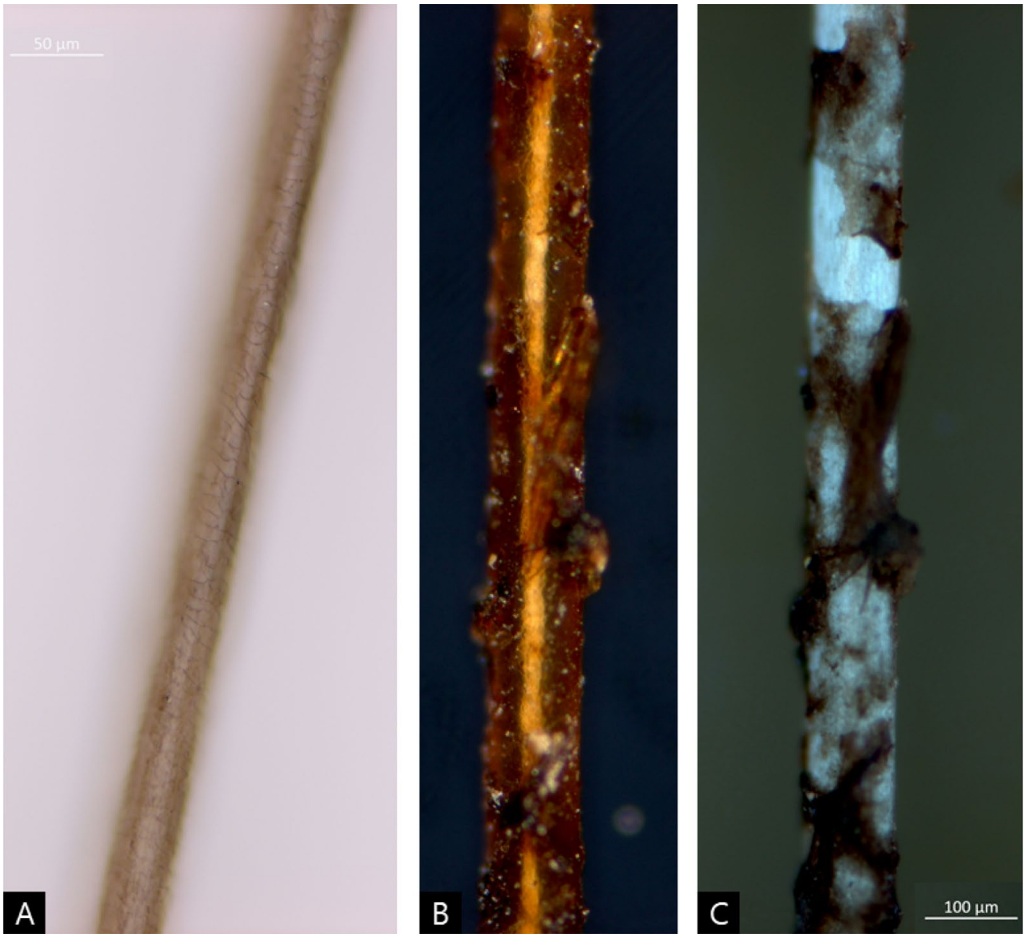

Fig. 8 Differences between human hairs retrieved from the breeches. A clean human (scalp) hair in good condition (**A**), in comparison to a soiled human (body) hair (**B**, **C**). UV light at 365 nm allows visual distinction of the hair shaft and the dirt covering its surface (**C**)

degraded, whereas keratinous animal fibers survive more easily. The microscopic differentiation of the recovered bast fibers is known to be difficult as the surface characteristics are highly similar [31]. While it appears yet unconventional, some studies suggest the use of spectroscopic methods to aid with this differentiation [32, 33]. For now, it is assumed that the textile remains originate from linen, based on the color impurity of the fiber bundles and the suggestions made by textile archaeologist S. Comis [1]. Given the degraded and soiled state of the textile remains, it is believed to be authentic.

The remaining samples contain various materials, among which a linen fiber (8.6), wood fragments (6.3), plant stem (8.5) and insect wings (4.6) as identified by the forensic experts. The other samples are believed to be of botanical origin. With an abundance of possible plant origins, the botanical class appeared to be most difficult to further subdivide, also due to the lack of identifying fragments (e.g., seeds, flower parts or leaf fragments).

Figure 9 shows one way of how all the different trace materials and clusters can be sorted into a classification system. Most traces consist of biological material. While sub-classes such as the animal and textile materials can be further specified, others remain at a higher level of classification due to the lack of characteristic features of the traces. The botanical sub-class is an example of this, whereas the hair samples provided enough details for further determination. An indication of possible contamination is also provided. Of the man-made materials, only the loose fibers could derive from contamination with the polyester threads clearly originating from a contamination event, as this material was developed in the twentieth century [34].

As stated before, half of the human scalp hair was in good condition, supporting the hypothesis that these strands originate from twentieth century individuals who have been handling the object and not from the buried individual himself or other whalers. For the materials

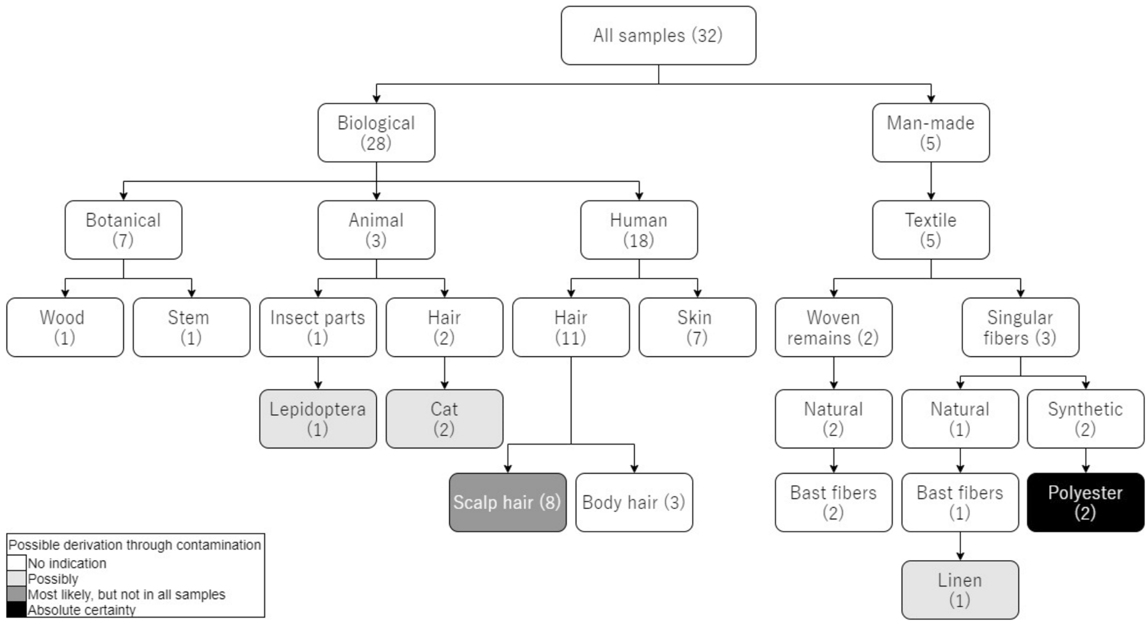

Fig. 9 Classification of the loose trace material found on and within the breeches. The trace material has been divided into two main classes: biological and man-made materials. Greyscales at the deeper levels indicate whether the trace material within that group could be the result of contamination, with the darker colors indicating a higher probability of external origin. The number of samples per (sub)class are indicated between brackets

from animal origin, contamination can also not be ruled out. However, the degraded state of much of the traces suggests that these materials indeed originate from the grave on Spitsbergen.

Discussion

The aim of the current study was to determine to what extent objective information can be gathered by conducting a forensic investigation on a pair of eighteenth century breeches in the Rijksmuseum collection. The classification and tentative identification of commingled and unidentified trace materials demonstrate the suitability of the forensic approach within cultural heritage and provides valuable information which can be used in decision-making regarding future conservation and display of the object.

A forensic investigation was conducted following official protocols and guidelines, supported by photography and newly created documentation forms in line with chain of custody guidelines. With a microscopy-based visual inspection, trace material was examined and classified through expert consultation. It appeared that a majority of the trace material, as expected, was of biological origin. With the combined use of SEM–EDX and ATR-FTIR, the presence of human material on the breeches was shown. Specific peaks that are indicative of lipids and protein were found in the ATR-FTIR spectra. These peaks are characteristic in the infrared (and Raman) spectra of human skin, as skin is rich in lipids and protein (mainly collagen and keratin) [35]. Comparison of the obtained ATR-FTIR spectra with those from a spectral database and results from other studies focusing on mummified skin further supported this conclusion [36–39]. These studies often had complete mummified remains at their disposal. The material of the current case study, however, was severely fragmented and unidentified. Yet, the findings indicate that the methods applied are still suitable under these conditions in line with other studies involving the recovery of human material from polar climates [12–14].

It was found that the forensic framework and associated document formats can be applied with minimal adjustments within cultural heritage research. In this case, existing documentation regarding the breeches themselves and especially the traces was very limited, which is a common issue in museum collections. By applying the correct documentation supported by detailed photographic record of the investigation or restauration of a museum object, the chain of custody within cultural heritage could be greatly improved with relatively limited effort. The forensic trace examination was found to be applicable to cultural heritage objects. Of course, museum objects demand extra care, and the forensic procedure should reflect this with a focus on non-invasive techniques. Due to the brittleness of the fragmented material, the forensic procedure regarding packaging was found to be suboptimal. To avoid further fragmentation, a deviation from the protocol was necessary and the samples were transferred from glassine envelopes to plastic tubes. Yet, this type of customization can also be found within forensic casework, where each crime scene is considered to be unique and might require tailor-made solutions to conduct the investigation in a proper, sustainable and valuable manner.

Modern forensic analysis techniques could be further optimized for the analysis of the severely degraded and soiled biological traces. This is expected since regular casework rarely involves items and trace material spanning such a large post-mortem interval as presented in the current study. It appears that there is no straightforward approach available for these severely degraded traces, which is further supported by the difficulty that forensic experts experienced when attempting to identify these traces. A more extensive collaboration between the fields of forensic science and cultural heritage could lead to interesting options for further improvement. Nevertheless, the current study succeeded in obtaining valuable information by determining the generic composition of the trace material.

A limiting factor of the current study is the lack of proper information management to avoid bias and limit subjectivity [40]. In a museum context, the segregation of roles and responsibilities as found within the forensic process is typically not present. Instead, the examiner is involved in every step and will have full access to the context information gathered in the assessment phase prior to the investigation. Being aware of potential pitfalls with respect to bias, the examiner should remain open-minded and as objective as possible and should define multiple propositions regarding the nature and origin of the object and associated materials. It was for this reason that the consulted experts received limited context information. However, the experts only received photographic material which could also limit their contribution. With access to the trace material and using their preferred equipment, perhaps a more detailed classification of the trace material could have been achieved.

Further classification of the trace material to more detailed sub-classes perhaps can also be achieved by using more destructive methods. The current study focused on less invasive methods to protect the integrity of the object and the associated traces, which is of high importance when working with museum objects. However, while a microscopy-based approach proved itself to be suitable to a certain extent, it may not be sufficient to characterize the trace material to the level of identification. Performing a destructive analysis on a minimal amount of trace material could therefore be considered. As botanical remains appear to be the hardest group to classify with sufficient confidence, these materials are of interest for follow-up research. This could include gas chromatography mass spectrometry (GC–MS) to investigate the presence of cellulose to confirm plant material [41], or palynological analysis to investigate the composition of pollen and support the geographic link between the Netherlands and Spitsbergen and indicate seasonality [42]. Furthermore, the wood on the backside of the breeches has not been sampled. Identification of this trace material may answer the question whether the wood has been brought from the Netherlands or has a different origin. More opportunities are also expected through the involvement of archaeology, where working with aged and degraded material is the standard. The development of a guideline on how to establish the nature and identity of unknown, aged trace materials in cultural heritage could be very useful as such a framework is currently not available.

With the identification of human material present in the commingled and fragmented trace material, the cultural heritage value of the breeches has been increased. The clear indication that these breeches have indeed been retrieved from a grave supports the history of the object and enriches the story that this object tells. The current study also confirms the observations made directly after the Smeerenburg project, although the presence of pubic hair was found to be very scarce [1]. Human nail was ruled out as the potential source of the trace material. Only a few traces were found to (most likely) originate from contamination which further adds to the value of the trace material. Despite the lack of precautions during the excavation and subsequent transport and handling of the item, the breeches have been handled with care leading to a limited degree of trace contamination. With this knowledge, a well-informed decision can be made regarding future conservation work and whether to remove material from the breeches.

Conclusions

A forensic approach can contribute greatly to the understanding of cultural heritage objects. The forensic documentation framework provides a systematic and formal manner to collect information, ensures that this information is traceable and provides a chain of custody of cultural heritage objects. Forensic trace analysis and identification resulted in the classification and in part identification of unknown biological trace materials.

The current study is one of the few to link the fields of forensic science and cultural heritage. It has demonstrated how the systematic forensic approach can successfully contribute to the objective assessment of trace material within a museum setting and that a mostly non-destructive approach yields valuable information as to aid the conservation process and display in the museum. Using this work as a starting point and an example, it hopefully will lead to more museum objects to be documented and analyzed using proven contemporary forensic methodologies.

Abbreviations
ATR-FTIR: Attenuated total reflection–fourier transform infrared spectroscopy; GC–MS: Gas chromatography–mass spectrometry; NFI: Netherlands Forensic Institute; SEM–EDX: Scanning electron microscopy–energy-dispersive X-ray; XRF: X-ray fluorescence.

Supplementary Information
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Additional file 1. Additional figures S1–S18 and additional tables S1, S2.

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Author contributions
YG, KK, SM and AA contributed to the conception and design of the work. YG acquired and analyzed the microscopic data. KK acquired the ATR-FTIR data and KK and YG performed the SEM–EDX measurements. YG interpreted the data obtained by ATR-FTIR and SEM–EDX. YG wrote the manuscript with the help of KK, AA and SM. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Netherlands Forensic Institute, P.O. Box 24044, 2490 AA The Hague, The Netherlands. [2]Rijksmuseum Amsterdam, P.O. Box 74888, 1070 DN Amsterdam, The Netherlands. [3]Van't Hoff Institute for Molecular Sciences, University of Amsterdam, P.O. Box 94157, 1090 GD Amsterdam, The Netherlands. [4]Co Van Ledden Hulsebosch Center (CLHC), Amsterdam Center for Forensic Science and Medicine, P.O. Box 94157, 1090 GD Amsterdam, The Netherlands.

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