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Ultrafast High-Resolution Mass Spectrometric Finger Pore Imaging in Latent Finger Prints

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Latent finger prints (LFPs) are deposits of sweat components in ridge and groove patterns, left after human fingers contact with a surface. Being important targets in biometry and forensic investigations they contain more information than topological patterns. With laser desorption mass spectrometry imaging (LD-MSI) we record 'three-dimensional' finger prints with additional chemical information as the third dimension. Here we show the potential of fast finger pore imaging (FPI) in latent finger prints employing LD-MSI without a classical matrix in a high-spatial resolution mode. Thin films of gold rapidly sputtered on top of the sample are used for desorption. FPI employing an optical image for rapid spatial orientation and guiding of the desorption laser enables the rapid analysis of individual finger pores, and the chemical composition of their excretions. With this approach we rapidly detect metabolites, drugs, and characteristic excretions from the inside of the human organism by a minimally-invasive strategy, and distinguish them from chemicals in contact with fingers without any labeling. The fast finger pore imaging, analysis, and screening approach opens the door for a vast number of novel applications in such different fields as forensics, doping and medication control, therapy, as well as rapid profiling of individuals.

Latent fingerprints (LFPs) are fundamental subjects in forensic sciences since the 19th century^{1–3}. The hands and feet are covered with a natural secretion due to the eccrine glands, which produce sweat, a mixture of water, salts, and other trace compounds^{4,5}. Various techniques have been used for analyzing latent finger prints including dusting with powders, spraying with reagent solutions for visual observation and spectroscopic techniques such as ultraviolet/visible, fluorescence, infrared and Raman spectroscopy^{6–10}. Successful identification of gender-specific biomarkers in volatile compounds of human sweat and peptides and proteins in LFP suggest the applicability of mass spectrometry imaging (MSI) for forensic application. Compared to other techniques, mass spectrometric (MS) methods offer the major advantage of chemically specific detection and identification of various endogenous compounds such as lipids and exogenous components from soaps, disinfectants, cosmetics, perfumes, drugs metabolites, and food residues in a single analysis. The presence and absence of these compounds can, in principle, be useful for forensic identification purposes and medical diagnostic and drug metabolite studies. Various ionization techniques such as matrix-assisted laser desorption/ionization mass spectrometry (MALDI), desorption electrospray ionization (DESI), surface-assisted laser desorption ionization (SALDI), and secondary ion MS (SIMS) have been applied for chemical imaging of LFPs^{11–17}. In general a resolution of 300 dpi is considered to be sufficient for the unambiguous identification of individuals. Separation of overlapped fingerprints due to their molecular differences is one major application in this field which facilitates the matching of images against the central fingerprint database^{18,19}. MSI of LFPs is still limited to level 1 and 2 features including arches, tented arches, loops, double loops, whorls, eyes, bifurcations, line fragments and endings of finger-lines²⁰. It has to be pointed out, that Level 1–2 features can be easily faked by simple reproduction techniques. Level 3 features, including pores, additionally carry significant discriminatory information for fingerprint matching and have further impact on investigative work with relevance in forensic sciences, for instance: age, ethnic origin, medication, psychological state, health, and metabolism^{4,5,20}. Up to the present no sweat pores have been resolved nor analyzed with MSI by SIMS where the resolution should be sufficient and better than any DESI- or MALDI-MS. The preservation of structural details of the fingerprint much beyond 50 μm to resolve sweat pores is hardly possible to achieve by DESI-MS and difficult to obtain with MALDI-TOF-MS sample



preparation employing a standard matrix with nebulizers. In this work, we have analyzed LFPs by high-spatial resolution MALDI-TOF-MS employing a thin gold-layer rapidly sputtered on top of the sample and an intelligent “search and scanning” approach to get a molecular insight into these level 3 features.

Based on the experiments we propose a sample preparation and scanning scheme combining optical sample analysis, image processing and spatially restricted mass spectrometry for the rapid molecular assessment of relevant LFP-features. It is clear that the resolution of finger pores and fast finger pore imaging, analysis, and screening opens the door for a number of unique novel applications in such different fields as forensics, doping and medication control, therapy, as well as rapid individuals profiling. The major bottlenecks for high resolution MSI scans beyond laser spot limitations are: i) very long data acquisition times, ii) data handling and processing due to the huge amount of generated MS spectra iii) loss of resolution and homogeneity during sample preparation, and iv) transfer and conservation of latent finger prints and localized pore excretions on a substrate. We have successfully circumvented the majority of these problems by employing a high resolution MALDI imaging system and avoiding a classical molecular matrix. We have instead used thin film laser desorption and ionization employing an Au-coating on top of LFPs using a fast magnetron sputter coating process. However, the main innovation is a fast optical scan of the visualized Au coated finger print with a desktop scanner and the computer controlled identification of finger pore coordinates. These coordinates are read out and processed and they are used to selectively probe and image finger pores with the combined high-resolution MALDI mass spectrometer. The complete preparation and MS recording procedure lasts a few minutes and is 2–3 orders of magnitudes faster than full-scans with MALDI imaging and generates only a limited amount of data from the most interesting areas.

Results and Discussion

In latent fingerprints the ion intensities are not homogeneously distributed over the complete contact area. A huge variety of substances not only exists between different donors (inter-person variation) but even at different positions of one finger tip⁹. Recently, it has been postulated that areas of high signal intensities may refer to skin pores²¹. In forensic sciences skin pores are attributed to level 3 features, which carry significant discriminatory information of a fingerprint²⁰. The diameter of skin pores is associated with the acrosyringium diameter of eccrine sweat glands and is in the range of $100\ \mu\text{m}^4$. These tiny structures are difficult to access by conventional MALDI-TOF-MSI for different reasons. General sample preparation techniques rely on time-consuming spray coating of a matrix-solvent mixture, which may influence the spatial resolution of soluble level 3 fingerprint features. Furthermore, common mass analysis of fingerprints proceeds in a rather low-resolution mode with large laser spot dimensions, and only level 1–2 features are recorded. For example, Tang et al. described laser spots with an elliptical shape of about $50\text{--}80\ \mu\text{m} \times 150\text{--}200\ \mu\text{m}$ and a spot to spot distance of $60\text{--}90\ \mu\text{m}^{19}$. We have analyzed a limited region of the fingerprint in a much higher resolution (laser spot to spot distance $25 \times 25\ \mu\text{m}$, spot dimension: $\sim 6\text{--}8\ \mu\text{m}$; see Figure 1). Molecular imaging of the signal attributed to K^+ ions revealed spherical structures of approximately $100\ \mu\text{m}$ inside the single ridges and may be attributed to skin pores (Figure 2a). By overlaying additional ion signals internal structures and chemical contrast of these spherical structures can be monitored and amplified. As estimated from Figure 2b, the density of these structures is about $400\text{--}500/\text{cm}^2$, which is similar to the quantity of sweat glands on palms ($370\text{--}700/\text{cm}^2$). The corresponding single mass spectra of the regions specified as a (“ridge area”), b (“pore frame”), and c (“valley area”) in Figure 2c are presented in Figure 2d. Only in the so-called “pore frame” region signals with m/z from $50\text{--}300$ are abundant.

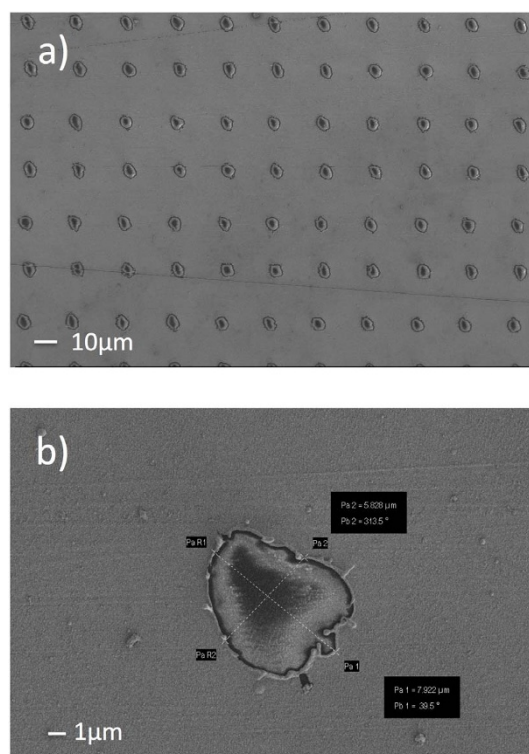


Figure 1 | Scanning electron micrographs of laser spots using the $25 \times 25\ \mu\text{m}$ resolution mode. The dimension of a single laser spot is <10 micrometers.

However, assignment of selected signals to physiological substances without any reference is rather challenging due to the complex composition of fingerprint residues and mass calibration issues in the low mass range. Abundant ions at m/z 23.4, 40.4, 82.5, 98.4, 112.3, 114.4, 128.5, 132.4, 134.5 may be attributed to Na^+ , K^+ , urea, Gly $[\text{M}+\text{Na}]^+$, Ala $[\text{M}+\text{Na}]^+$, Gly $[\text{M}+\text{K}]^+$, Ser $[\text{M}+\text{Na}]^+$, Leu $[\text{M}+\text{H}]^+$, Asp $[\text{M}+\text{H}]^+$.

As emphasized above, MSI of a complete fingerprint, especially in a high resolution mode, is a time consuming procedure and a lot of analytical data has to be handled. Thus, we were interested in a simplification and acceleration of the overall analytical process. Recently, latent fingerprints were visualized by gold sputtering and analyzed by MALDI-TOF-MS¹⁹. The visualization of latent fingerprints was attributed to the absorption of visible light by surface plasmon resonance (SPR) of the formed gold nanoparticles. Herein, we have used a related sample preparation process (very thin layer instead of deposition of nanoparticles) to visualize pores and sweat coming out of the pores by “stimulated heating” (Figure 3). In principle, the coordinates of active pores from a pore print (Fig. 3a) as obvious as purple spots on a gold nano-layer can be determined automatically by image processing software and converted (Fig. 3b) in order to direct the movable sample table of the mass spectrometer. Thus, sample preparation for both-, mass and optical analysis can be achieved in one single procedure, which can be accomplished in a few minutes. Mass analysis of only a few pore spots may allow for the identification of endogenous compounds and distinguish them from exogenous ones. As a consequence, the identity and the chemistry of a fingerprint can be accessed within an extremely short timescale by fast optical analysis of a complete and mass spectrometry of the most important regions of a prepared fingerprint, which can be downsized to a one- (or few) spot analysis. The general principle is shown in Figure 4. It has to be mentioned, that combined optical and mass spectrometric analysis may not be limited to the analysis of pores and can be expanded to other level 1–3 segments of a fingerprint. Thus, a

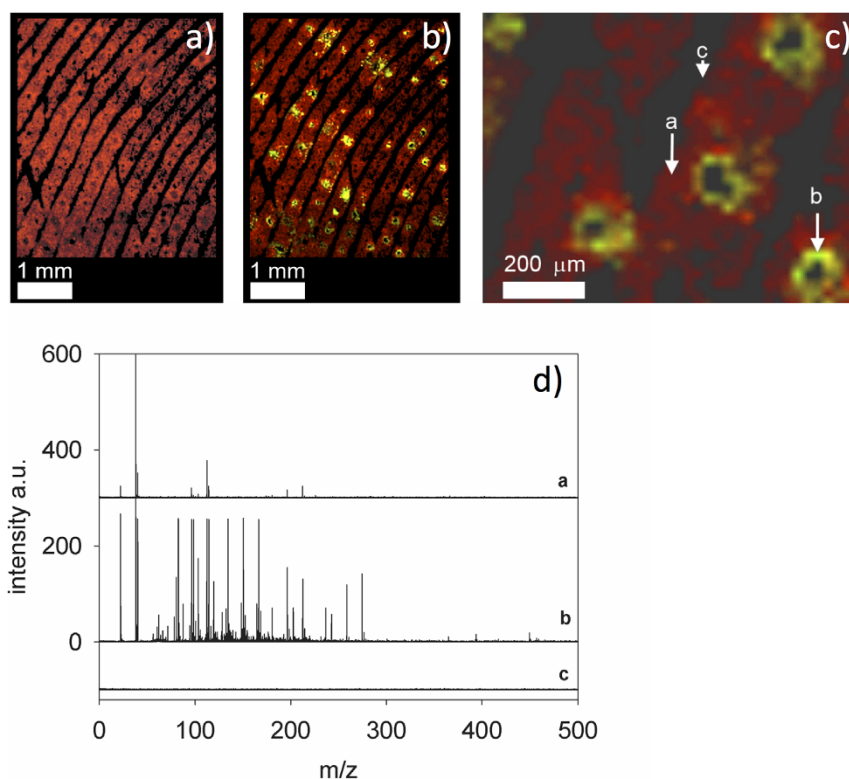


Figure 2 | High-spatial resolution MSI of fingerprints displays pores inside of ridges by m/z signals attributed to K^+ (a, red) and overlaid signals (b) of m/z 103 (green) and 166 (yellow). Single laser spot mass spectra of regions specified in (c) are presented in (d).

similar process for the rapid analysis of overlapped fingerprints by iterative one-spot MS analysis and computer aided reconstitution of fingerprint segments based on more sophisticated optical object analysis algorithms appears feasible.

We have tested the opportunity of one-spot MS analysis on a selected drug example of loratadin by treatment of a voluntary test person. Loratadin is an antihistamine drug, which is used for the symptomatic therapy of allergic coryza and idiopathic urticaria and has a plasma half life of 8 hours. After oral medication it will be absorbed quickly and metabolized to desloratadin. Plasma peak concentrations of loratadin and desloratadin appear 1–1.5 and 1.5–3.7 hours after application, respectively²². Skin emitted loratadin has recently been analyzed by DESI mass spectrometry and could be clearly detected 50 minutes after oral consumption of 10 mg²³.

Oral medication of loratadin (20 mg) and sample preparation by sweating of a pre-cleaned finger in a plastic bag 4 hours after consumption provides analysis of endogenous loratadin within active pores. Signals, which can be assigned and attributed to loratadin from a co-spotted reference sample (loratadin tablet), were clearly found in the fingerprint. In addition, significant signals which are assigned and attributed to metabolized desloratadin were only found in the fingerprint and are absent in the reference (see Figure 5). It may be postulated that the proportion of the drug signal to the metabolite signal corresponds to the application time of the drug, which may be of interest in special medical or forensic cases. Indeed we have found a drug/metabolite ratio of about 1 : 10 which roughly corresponds to the ratio of the plasma concentrations after 4–6 hours.

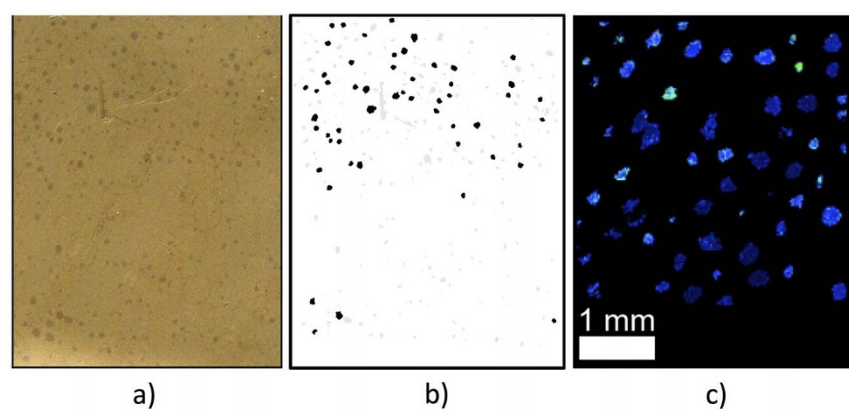


Figure 3 | Visualization of active pores of a prepared fingerprint on a gold-coated polyethersulfone membrane after finger cleaning, sweating, deposition, and coating the LFP with a 5 nm gold-layer. The image was taken using a commercial desktop scanner (a) and further processed in ImageTool 3.0 for object analysis (b). The corresponding MSI (high resolution section of a)) displays the spatial intensity distribution of m/z 112 attributed to alanin $[Ala+Na]^+$ after a full scan of the fingerpore print (c).

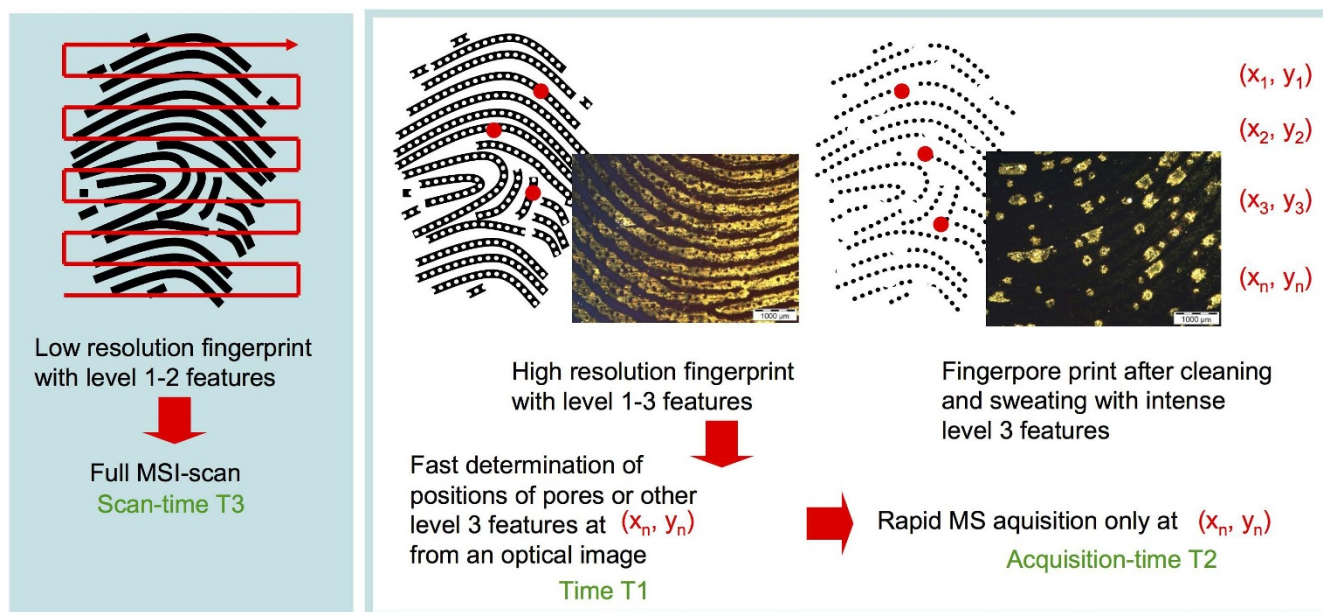


Figure 4 | Principle of fast Au-coated fingerpore imaging (right) in comparison to full MSI-scans (left) of a fingerprint. Note that $T_1 + T_2 \ll T_3$.

Conclusions

We have shown that fast finger pore imaging, analysis, and screening opens the door for unique applications in forensics, doping and medication control and disease therapy. The high mass resolution in principle enables the resolution of isotopes and isotope distributions. At a suitable dynamic range and the absence of interferences of mass signals they may give an intriguing possibility for rapid individuals profiling on region or ethnic specific markers in the near future. The main innovation is the development to mitigate or circumvent the problem of time consuming complete high-resolution fingerprint MS scans to obtain combined topological and chemical information. The fast optical scan of a complete fingerprint after rapid (vacuum) deposition of a nanometer-thick gold layer enables the rapid identification of regions of interest, e.g. finger pore coordinates, manually or automatically by image processing. Single spot mass scans on the selected coordinates probe the chemical constitution of fingerprints only in these most interesting regions. Thus, the combined optical and mass spectrometric procedure is significantly faster than full

fingerprint scans with MALDI imaging allowing in principle for a detailed analysis of inter/intra-person and finger variations of fingerprint constituents. Moreover, it has been shown that maintaining high-spatial resolution even during sample preparation and laser desorption is necessary to obtain topological and chemical information of level 3 features by high resolution MALDI-TOF-MSI of LFPs. Since level 1–2 features can be easily faked, for instance, by the generation and use of artificial skin imprints, the analysis of the spatial chemical distribution of level 3 features discloses imitations. Furthermore, the introduced method allows for the rapid analysis of endogenous compounds via the optical identification of skin pores and the mass analysis of the secreted sweat therein. In summary, the combination of image analysis and mass spectrometry of selected regions after simple sample preparation appears to be highly promising to expand the application area of mass spectrometry even in investigative forensic and medical sciences, as well as in nearly real time security observations with respect to the examination of detail rich level 3 features.

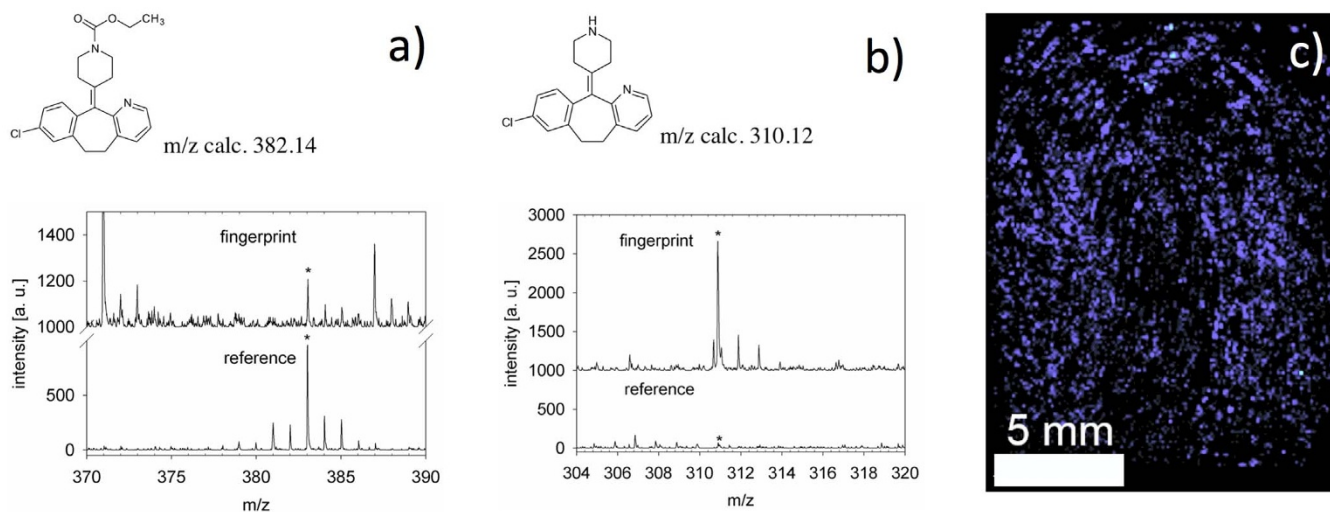


Figure 5 | Single spot MALDI-TOF-mass spectra of the drug loratadin (a), the corresponding metabolite (b) and the spatial distribution of m/z 383 attributed to loratadin ($[M+H]^+$) taken from a fingerprint after cleaning and sweating, coated with a few nanometer Au-layer (c).

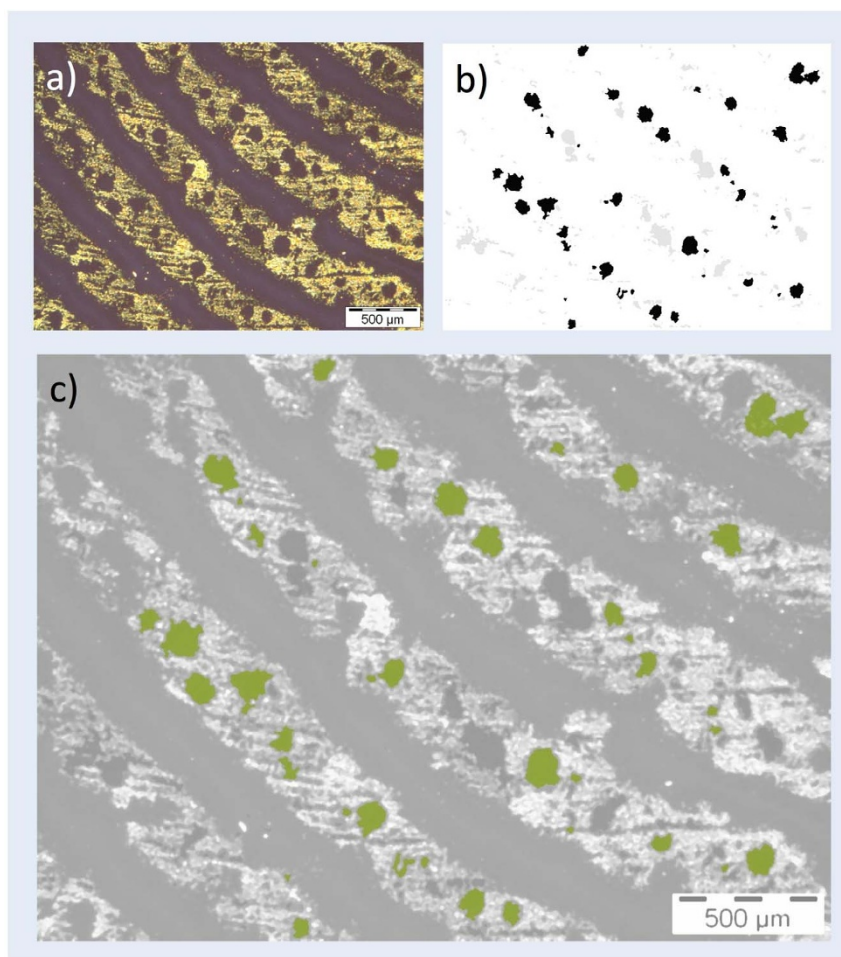


Figure 6 | Example of ImageTool 3.0 object analysis from an optical microscope image of a gold-coated fingerprint. (a) Original fingerprint (optical microscope image), (b) found and attributed (black) and rejected (grey) objects, (c) a false colour overlay of (a) and (b).

Methods

Preparation of fingerprint-prints. The finger was cleaned with ethanol (HPLC gradient grade)-water (Millipore grade)-ethanol using a soaked tissue (KIMTECH science precision wipes 05511-7552, Kimberly-Clark Europe Ltd., United Kingdom) and the hand was placed into a plastic bag avoiding surface contact and heated with an IR-lamp (Philips R95E) for 10 minutes to stimulate the secretion of sweat through the pores of the skin. Latent fingerprints of voluntary test persons were prepared on gold-coated substrates by gentle pressing such a prepared or original finger against the substrate (glass slides, membranes (Millipore Express plus cat# GPWP14250, Merck KGaA, Germany) for about 2 s.

Gold-sputtering. For the vacuum deposition of gold the substrates (glass slides, membranes) were placed into the chamber of an Auto 306 magnetron sputter coater (Edwards Ltd., United Kingdom) and transferred into the plasma zone by the use of a shutter system as the operating pressure was stable at 2×10^{-2} mbar. High-purity argon gas (5.0 premium quality, Fa. Air Products) and a high-purity gold target were used. The coater was operated at 30 W and a deposition rate of 0.24 nm/s. The sputter conditions were adjusted for the deposition of a 30 nm base gold layer on the substrates and a 5 nm gold layer on top of the LFP on the substrates after their generation described previously. The distance between the substrate and the target was 115 mm.

Imaging mass spectrometry. Imaging mass spectrometry was performed on a Autoflex III mass spectrometer equipped with a Nd:YAG SmartBeam® laser (355 nm, Bruker Daltonic, Bremen, Germany). The samples on the slide were mounted via a MTP Slide Adapter II (Bruker Daltonic, Bremen, Germany). The experiments were performed in reflectron mode by the use of instrumental parameters as follows: Ion Source 1: 19.11 kV, ion source 2: 16.65 kV, lens: 8.74 kV, reflector: 21.09 kV, reflector 2, 9.75 kV, detector gain: 1.0–5.0, sample rate 2.0, shots: 10, repetition rate: 200 Hz, laser attenuator: offset: 75%, range: 20%, set: 1_minimum, fine adjustment: 60%. The mass spectra were acquired in the m/z range up to 1000 without deflection. The instrument was controlled by the FlexControl 3.0 and FlexImaging 2.1 software (Bruker Daltonic, Bremen, Germany). Data were further processed in FlexImaging 2.1.

Semi-automated spatially resolved single spot mass analysis (see Figure 6). After gold-layer deposition on-to a fingerprint an original image was taken with an EPSON PERFECTION V300 PHOTO desktop scanner at 1200–2400 dpi (jpg- or tif-file format) and further processed in UTHSCSA ImageTool Version 3.0²⁴ using the integrated script language (itm-file format) for object analysis. Relevant objects were generated by the “FindObjects”-command after employing some picture conversion and filtering functions. Objects were analyzed by the integrated plugin “objanal” and sorted out concerning several parameters like area, minor and major axis length, and elongation. Centroid- (x, y)- coordinates of the relevant objects were exported as `<Spot Pos = "x,y"></Spot>` commands which were manually copied into the msi-file generated in FlexImaging (BRUKER, Germany) on the original image. Mass analysis was performed using the mode “Tissue Profiling” in FlexImaging 2.1. Teaching of the substrate was performed manually by the use of laser engraved or printed micro-features. The script (short program) for FPI-data recording and analysis can be obtained free of charge from the authors upon request.

Conservation of latent finger prints. Latent finger prints can be transferred from the substrate/surface with and conserved on a soft gold sputtered soft membrane (Millipore Express plus, Merck KGaA, Germany).

Statement of the authors and permission to use data from human individuals. The authors confirm that all experiments (taking finger prints of a volunteer/individual) were performed in accordance with relevant guidelines and regulations of the *Leibniz Institute of Surface Modification in Leipzig* (Managing board), Germany, the *Leibniz Society*, and the *German Science Foundation (DFG)* about experiments with humans and data treatment and protection of personalized data. All experimental protocols as well as publication of the data were finally approved by the institutional and licensing committee, as well as the executive board of the *Leibniz- Institute of Surface Modification in Leipzig*. An explicit informed consent was obtained from the anonymous volunteer providing the finger prints. The individual explicitly allowed the authors to use the data in the present publication.

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Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. B.A. and C.E. designed the experiment. C.E. performed the experiments. All authors discussed the results and wrote the manuscript.

Additional information

Competing financial interests: Yes, there is competing financial interest. A patent has been filed on the technology reported in the paper. PCT application in preparation (DP 10 2013 017 649.2).

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