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Forensic Identification of Gender from Fingerprints

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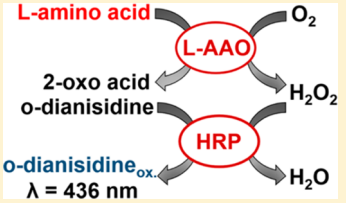
ABSTRACT: In the past century, forensic investigators have universally accepted fingerprinting as a reliable identification method, which relies mainly on pictorial comparisons. Despite developments to software systems in order to increase the probability and speed of identification, there has been limited success in the efforts that have been made to move away from the discipline's absolute dependence on the existence of a prerecorded matching fingerprint. Here, we have revealed that an information-rich latent fingerprint has not been used to its full potential. In our approach, the content present in the sweat left behind—namely the amino acids—can be used to determine physical such as gender of the originator. As a result, we were able to focus on the biochemical content in the fingerprint using a biocatalytic assay, coupled with a specially designed extraction protocol, for determining gender rather than focusing solely on the physical image.



In modern criminology, fingerprints play a major role in forensics as a means of identification.[1] In the past 110 years,[2] the development of fingerprint analysis has stalled at simple visual comparison and matching, even though fingerprints—as samples of biological origin analogous to other body fluids—have the potential to provide much more information. Currently, the shape, size, and unique patterns associated with fingerprints are compared using various computational programs, such as Automated Fingerprint Identification System (AFIS). However, the ultimate setback is the requirement of matching fingerprints to be stored in a database or for the person of interest to be physically present for comparison. If neither of these conditions is fulfilled, the print is reduced to merely exclusionary evidence,[3] despite being stored in a separate database for future use when comparing them with new, incoming fingerprints. The same can be said about DNA analysis. Even though DNA can provide potentially the most significant information about the fingerprint originator, DNA analysis can take weeks or months to be processed, and if there is no matching DNA profile in the database, then the potential for information is significantly reduced. The purpose of our approach is to address the issue of a fingerprint not having an immediate matching image or DNA profile. While the patterns of individual fingerprints, as well as DNA, act as important sources of evidence, it is often overlooked that sweat and sebum are also left behind with a fingerprint. Sweat contains varying amounts of metabolites produced by the body depending on various processes related to metabolism. Metabolism, which is directed by a combination of multiple hormone-based control mechanisms,[4] acts as a function of physical properties such as gender, age, ethnicity, or health status. It is also known that the amino acid content of an individual can slightly vary depending on the physiological state of the individual's metabolism. This slight variation occurs over time scales of several hours and can also be somewhat affected by certain medications[5,6] as well as after the consumption of certain foods. It has been found that amino acid levels differ between people in different demographic groups such as gender (i.e., male and female).[6−8] As a result of this difference, the biological/biochemical content from the sweat left behind with fingerprints can be exploited, in a method similar to the way clinical diagnostics uses other body fluid contents,[9−12] to gain valuable information on different "persons of interest" connected to a particular crime scene.

In fact, research has already started in this area using spectrometric methods such as matrix-assisted laser desorption ionization (MALDI) and liquid chromatography−mass spectrometry (LC−MS) as well as spectroscopic methods including infrared and Raman spectroscopy.[13] Two examples of this research involve the use of desorption electrospray ionization (DESI) mass spectrometry to detect various explosive-related compounds[14] and Raman spectroscopy to detect drug compounds in the secretions left behind with fingerprints.[15] At the moment these methods require rather sophisticated instrumentation that do not always allow for on-site analysis, despite their incredibly selective and reliable nature. These devices also demand highly specialized personnel that are not likely to be available as part of the immediate forensic investigation team. In addition, recently published efforts using nanostructured materials and affinity-based techniques aim for the detection of compounds such as cotinine[16] and THC[17] in the latent fingerprint content. Notably, there has been progress in the use of various instrumental studies on latent fingerprints as demonstrated by the Kazarian group. They have performed studies in spectroscopic[18] and chemical imaging[19] of latent fingerprints. The possibility of using the endogenous compounds in fingerprints for the determination of various personal attributes has also been noted by other instrumental chemists.[20] The vast majority of these methods, however, can only focus on the presence or absence of certain

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chemical compounds. While this can be useful, there is still a limit to the conclusions that can be drawn based on these results.

Despite the success in differentiating between genders using ridge density, a complex statistical analysis as well as visual comparison by counting the number of ridges present in a 25 mm^2 area that are present is still required.[21,22] We have recently demonstrated that bioaffinity-based analytical systems are able to distinguish between African-American and Caucasian ethnicities by analysis of biomarkers commonly present in blood.[23] This was followed by a similar study that showed it is also possible to distinguish between males and females.[24] A very recent example[25] of the success of these bioaffinity systems involves the parallel analysis of two blood markers in order to determine age of the blood sample found at a crime scene. Because the contents of fingerprints can provide information in a way that is analogous to the contents of body fluids, it should also be able to provide information similar to the previously mentioned study. In this work, the proposed methodology utilizes the bioaffinity interaction between an enzyme and its ligand (for example, substrate, cosubstrate, activator, inhibitor, etc.) in order to generate a visible color change that can be seen by the naked eye or spectroscopically quantified. The extraction/bioassay system presented here is the first proof-of-concept example of a system that can detect a physical characteristic of the fingerprint originator (male/female origin) based on the chemical content of the fingerprint, ignoring the traditional pictorial approach.

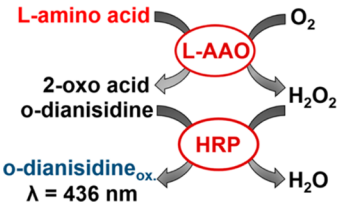
EXPERIMENTAL SECTION

Ethics Statement. The Institutional Review Board, Office of Pre-Award and Compliance at the University at Albany has fully approved the experimental protocols demonstrated in this manuscript. These protocols were carried out in accordance to the office's requirement of obtaining informed consent, in the form of a signature from each volunteer, acknowledging that they are aware of the procedure that will take place, any risks or benefits that may accompany the study, as well as acknowledging that they will not receive any payment for their participation. Informed consent from all volunteers who participated in this research study was obtained.

Enzymatic Assay Components. The following enzymes and organic/inorganic chemicals were purchased from Sigma-Aldrich: L-amino acid oxidase type IV (L-AAO, E.C. 1.4.3.2), horseradish peroxidase type VI (HRP, 1.11.1.7), *o*-dianisidine, (+)-sodium L-ascorbate, triethanolamine (TEA), L-aspartic acid, L-threonine, L-serine, L-glutamic acid, L-asparagine, L-glutamine, L-cysteine, L-proline, glycine, L-alanine, L-valine, L-cystine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, *β*-alanine, L-ornithine, L-lysine, L-tryptophan, L-histidine, L-arginine, and L-citrulline. Water used in all of the experiments was ultrapure (18.2 MΩ·cm) water from PURELAB flex, an ELGA water purification system.

Detection of Amino Acids. The dual-enzyme cascade, displayed in Scheme 1, was designed and optimized in the present study and realized in pH 7.6 TEA buffer containing 20 mU of L-AAO, 3 U of HRP, and 85 μM *o*-dianisidine. The cascade is activated when L-AAO reacts with a range of concentrations of amino acids present in the sample, which results in the conversion of O_2 to H_2O_2. The HRP then consumes the H_2O_2, causing the oxidation of the dye, *o*-dianisidine, present in the system. This results in the formation of the oxidized form of *o*-dianisidine, which is observed

Scheme 1. Dual-Enzyme Cascade Assay Containing L-AAO and HRP Used for the Differentiation of Gender via Fingerprint Content[a]



[a]The abbreviations used are L-AAO (L-amino acid oxidase) and HRP (horseradish peroxidase).

spectroscopically at λ = 436 nm. The intensity of visible color production is then proportional to the amino acid concentrations present in the sample. This enzyme cascade was first optimized using mixtures of the average amino acid concentrations specific for males and females, respectively. The reactions and optical measurements were performed at 37 °C using a SpectraMax Plus384 (Molecular Devices, CA) microplate reader with polystyrene (96 well) microtiter plates. The signal corresponding to the concentration of oxidized *o*-dianisidine was measured optically at λ = 436 nm.

Statistical Analysis. R-project software[39,40] was used to generate randomized concentrations of each of the amino acids found in fingerprint content to create 50 amino acid mixtures, with 25 mixtures representing male samples and 25 mixtures representing female samples. The L-AAO/HRP biocatalytic assay was then performed using the 25 mimicked male and 25 mimicked female fingerprint samples. Receiver operating characteristic (ROC) analysis was used to evaluate the performance of the assay and estimate the probability of distinguishing between the male (25 samples) and female (25 samples) groups of human fingerprint content. Using ROC analysis, the threshold (above which the absorbance changes correspond to the female group) that yielded the maximum accuracy was determined. ROC analysis involves changing the threshold and observing the effect on the predictive power of the model to produce an ROC curve. The area under the ROC curve (AUC) is a single measurement that summarizes the overall discriminating ability of the assay. It represents the probability that the diagnostic test will correctly distinguish between the male and female samples. The larger the AUC, the higher the probability that each sample will be identified correctly. Lastly, to demonstrate the viability of this method, real fingerprints were analyzed using the bioassay.

Amino Acid Extraction from Authentic Fingerprints. For the purpose of determining the viability of the biocatalytic assay when used with authentic samples, real fingerprint samples were collected from volunteers and the amino acids were extracted with a new protocol that was developed during this project. This protocol combines the use of an elevated temperature and acidic conditions to extract the water-soluble amino acids from the lipid-based content of the fingerprint, which is composed of triglycerides, wax esters, free fatty acids, and squalene.[26] The aqueous phase containing the amino acids was removed from the fingerprint surface and subsequently analyzed by the proposed biocatalytic assay. The extraction process consisted of the following steps: the fingerprints were deposited onto a portable surface composed of polyethylene and 120 μL of 0.01 M HCl was placed directly onto the fingerprint, covering an area of 63 mm^2. The entire surface was

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then heated at 40 °C for 20 min. This process causes the amino acid content in the fingerprint to migrate from the lipid-based content into the aqueous acidic solution, while the lipid-based content remains on the portable lipophilic surface. The aqueous acidic solution was then collected off of the portable surface and used as the sample for analysis.

RESULTS

Gender Determination via Bioassay. Here, we are proposing a bioaffinity-based sensing system that has the capability to distinguish between fingerprint samples from males and females using the concentrations of amino acids present in fingerprint content. The logic for this determination is based on the proven fact that females have different concentrations of amino acids in their systems than males.[7,27−31] The L-AAO enzyme has the ability to use a large range of L-amino acids as substrates, with a varying degree of affinity,[32−34] which drives the enzyme to convert oxygen to peroxide. L-AAO has previously been used as an amperometric biosensor[35] for the identification of amino acids as well as in an assay for detecting intestinal peptide hydrolase activity.[36] The peroxide then acts as a substrate for the secondary element of the cascade, HRP, which oxidizes a dye acting as a cosubstrate, thus, producing a signal at a particular wavelength. The dye used in this case was *o*-dianisidine, which becomes oxidized by HRP and is consequently detected at 436 nm.[37] As shown below, this method, combined with the newly developed extraction protocol allowing for the isolation of water-soluble amino acids from the lipid-based fingerprint content, requires only a minuscule amount of substrate. More importantly, it provides a quick male/female response and can be performed on-site. These results can narrow down the possibilities in a suspect pool in a quick and timely manner when there is no matching fingerprint image or DNA profile in the corresponding databases. Furthermore, this type of analysis can potentially be utilized by any and all members of law enforcement with no need for specialized training, as it works in a similar manner to pregnancy strips or glucometers.

Statistical Analysis of Mimicked Samples. The distribution of amino acid concentrations in human fingerprint content was previously studied,[27−31] and a list of the corresponding average amino acid concentrations can be found in Table 1. In order to investigate the distribution of and variability in amino acid concentrations found in fingerprint content, the influence of gender was examined. The studies, referenced above, reported significant differences in the concentrations of certain amino acids when comparing genders. The reported data were used in the present study to prepare solutions mimicking the levels of all amino acids present in the fingerprints of different genders.[8,28−31,38]

The first step of our study began with the statistical analysis of the available data from previous studies. The values were not normally distributed, but rather positively skewed and consistent with a log-normal distribution. The parameters of the log-normal distribution were available only for overall amino acid concentrations, while the distribution parameters estimated from the male and female data came from logarithmic untransformed data. The available parameters for a normal distribution were first corrected for a log-normal distribution. For each of the 23 amino acids present in the fingerprint content, random values according to the recalculated parameters of the log-normal distribution in males and females were generated using R-project software.[39,40] Thus, two

Table 1. Average Amino Acid Concentration (mM) Values for Males and Females Derived from Sweat[a]

AA	female conc. (mM)	male conc. (mM)
Thr	0.2090	0.1121
Ser	0.9840	0.5208
Glu	0.1780	0.1109
Gly	0.6463	0.3418
Ala	0.3870	0.1968
Cit	0.1967	0.1267
Asp	0.1196	0.0638
Asn	0.0380	0.0161
Gln	0.0178	0.0120
Pro	0.0728	0.0349
Val	0.0919	0.0459
Cys	0.0012	0.0009
Met	0.0085	0.0034
Iso	0.0494	0.0229
Leu	0.0625	0.0324
Tyr	0.0559	0.0303
Phe	0.0378	0.0172
β-ala	0.0128	0.0034
Orn	0.1361	0.0684
Lys	0.0528	0.0285
Trp	0.0151	0.0071
His	0.1790	0.0804
Arg	0.0948	0.0540

[a]These values have been previously reported and were used to prepare mimicked fingerprint samples.

sets of concentration values (25 for males and 25 for females) of all 23 amino acids were produced and randomly grouped together. This allowed for the concentration groups to capture a range of concentrations that amino acids can take in males and females. Then 25 experiments using these concentration groups were performed for each gender.

After obtaining the optical responses for the model solutions, seen in Figure 1, the measured output signal was defined as the

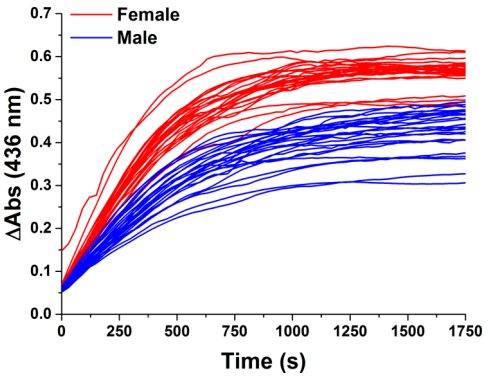

Figure 1. Change in signal response (λ = 436 nm) corresponding to the oxidation of *o*-dianisidine upon reaction of the analytical system. The top red traces indicate the female samples, and the bottom blue traces indicate the male samples.

absorbance of the oxidized dye as a function of time, once the reaction was initiated. The optical responses were then used for further statistical analysis. The bottom part of the graph corresponds to the male samples (lower concentrations of the amino acids) while the top part of the graph agrees with female samples (higher concentrations of the amino acids). The

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produced responses, which relate the absorbance to the concentrations of all 23 amino acids, are clearly different between males and females, with a small overlap between them.

The readout time was initially set to 420 s; however, after repeating the statistical analysis using later readout times up to 1800 s, the final result remained almost unchanged. The amino acid concentrations were chosen to follow the published distributions that are relevant for males and females; thus, real applications should generate signals that correspond to the signal distribution of the mimicked samples.

The area under the ROC curve,[41] also known as AUC, was estimated by the trapezoidal integration method, and the corresponding 95% confidence interval (CI) was estimated using the method described by DeLong et al.[42] The AUC was estimated at 0.99 (95% CI, 0.98−1.00) from the ROC curve, Figure 2, which means that the L-AAO/HRP assay has a 99%

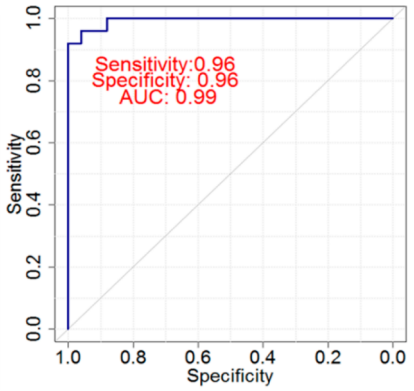

Figure 2. Trade-off between sensitivity and specificity is shown by presenting data as a receiver operating characteristic (ROC) curve. Area under the ROC curve (AUC) is 99%, which is the probability for the presented assay to correctly distinguish between males and females based on the amino acids' concentrations in the respective fingerprint samples. The optimum cutoff point was chosen with a sensitivity and specificity of 96%. Random choice is denoted by the gray diagonal line.

probability of correctly differentiating between male and female fingerprints. The ROC curve was generated from absorbance changes, and the best absorbance threshold of 0.439, which balanced the trade-off that exists between sensitivity and specificity, was determined. The absorbance change is the most accurate cutoff point for discrimination between male and female fingerprint samples. As shown, ROC analysis has proven the potential of this assay to correctly differentiate between samples from males and females.

Evaluation of Authentic Fingerprint Samples. The success in distinguishing between genders utilizing the bioassay explained above on the mimicked samples led to further studies involving authentic latent fingerprints. These fingerprints were collected from two groups of volunteers, Caucasian males and Caucasian females. The fingerprints were collected on polyethylene film according to an established procedure described by Croxton et al.[8] Once the fingerprints were deposited, the extraction protocol, detailed in the Experimental Section, was applied. Following the extraction, the amino acid samples were subjected to the same bioassay as the mimicked samples. As anticipated, the aqueous solution removed from the fingerprints contained the amino acids necessary for analysis, while the nonpolar content remained on the polyethylene film. The success of the extraction of amino acids as well as the bioassay's performance is demonstrated in Figure 3. As seen here, the samples from the authentic fingerprints generated a noticeably lower optical signal than that of the mimicked samples (Figure 1), which is a result of the dilution that occurs during the extraction of the amino acids from the authentic fingerprints. However, despite the difference in signal intensity, there is still a significant difference between the samples obtained from males and the samples obtained from females. The results of the L-AAO/HRP assay using authentic fingerprints were consistent with the results of the L-AAO/HRP assay using mimicked samples in that the female samples generated a significantly higher optical signal than that of the male samples, corresponding to the higher amino acid content. Additionally, the relative standard deviation between the signals produced by the authentic fingerprint samples, both left and right thumbs, does not exceed 8%.

Evaluation of Various Extraction Surfaces. Following the identification of gender from the authentic fingerprint samples, the extraction protocol and biocatalytic assay were further tested on various surfaces that could be found at a crime scene. Given that authentic male fingerprints did not generate a significant signal, only female fingerprints were used for this experiment. Three female fingerprints were deposited onto multiple surfaces including a door knob, a laminate desktop, a composite benchtop, and a computer screen. The polyethylene film used in the previous experiment for the determination of gender was used to remove the fingerprints from the respective surfaces. The samples were then subjected to the same extraction protocol, described above, as well as the L-AAO/

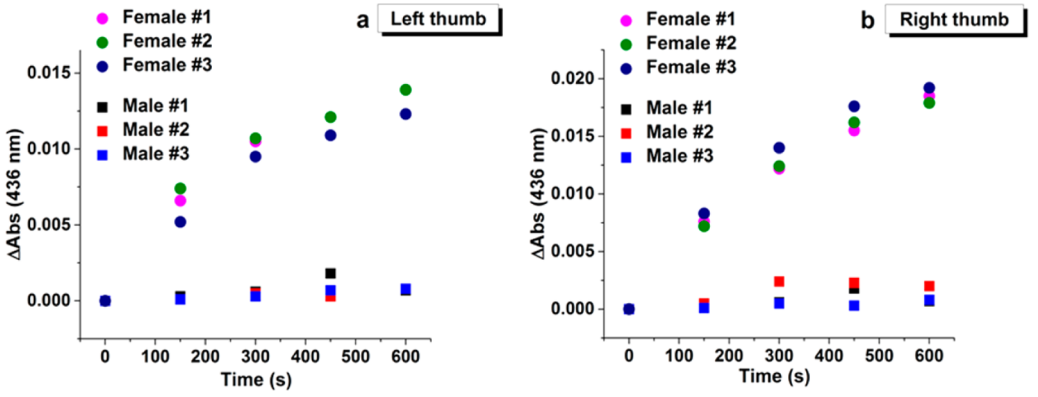

Figure 3. Data obtained from authentic fingerprint samples from Caucasian males and females: (a) the left thumb and (b) the right thumb.

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HRP bioassay. Figure 4 demonstrates that the extraction protocol and the bioassay are capable of identifying a female fingerprint, regardless of the surface from which it was taken.

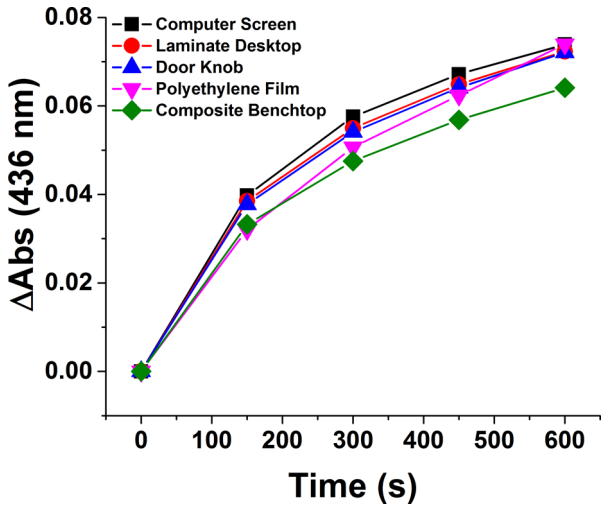

Figure 4. Female fingerprints (n = 3) taken from five different surfaces. Inset bar diagram represents the change in absorbance at λ = 436 nm, after 600 s of assay completion. Surfaces A–E in the bar diagram represent a computer screen, laminate desktop, door knob, polyethylene film, and a composite benchtop, respectively.

CONCLUSION

The L-AAO/HRP bioassay described above for the purpose of distinguishing between fingerprint samples obtained from both males and females has proven to be reliable and reproducible. The ROC analysis conducted using 50 mimicked fingerprint samples generated statistics proving that it is possible to determine the gender of the fingerprint originator using this method. Initial experiments using mimicked fingerprint samples, which were created and analyzed by statistical methods, concluded that there was a 99% chance of determining the correct gender of the fingerprint originator. Furthermore, a reliable sample extraction protocol was employed for the extraction of the necessary substrates—amino acids—from real fingerprint samples collected from Caucasian male and female volunteers. In the case mentioned above, the thumbprints of the volunteers were deposited onto a portable polyethylene film, where they were subjected to acidic conditions. The amino were then separated from the lipid-based components through heating the polyethylene film. The results from the analysis of authentic fingerprint samples further demonstrated the ability of the bioassay to differentiate between male and female fingerprint samples based on the significant difference in absorbance intensities. In addition, the durability of the bioassay and extraction process was successfully determined using various surfaces from which the fingerprints were collected.

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Notes

The authors declare no competing financial interest.

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