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Final Summary Overview
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Evaluating the skin microbiome as trace evidence

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Due to a move from CU Boulder to UC San Diego, this grant started October 1, 2015.
+Co-PI Metcalf joined the faculty at Colorado State University in Fall of 2016.

PURPOSE

The purpose of this project was to characterize basic transfer properties of an individual's unique skin microbial community to common surface materials, and to assess whether it is possible to characterize an individual's skin microbial handprint after a person is deceased. We had the following specific objectives in mind.

Objective 1: Determine whether the *sequence* in which surfaces are touched by the same person influences the detection of an identifiable skin microbial signature.

Objective 2: Determine whether the *number of times* a surface is touched by the same person influences the detection of an identifiable skin microbial signature.

Objective 3: Determine whether individual skin microbial signatures are recoverable after multiple people have touched an object.

Objective 4: Determine the stability of microbial signatures on surfaces over time.

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Objective 5: Determine the magnitude of change in microbial skin communities during morgue cooler storage (relative to initial samples at death scene).

PROJECT SUBJECTS

Subjects consisted of adult volunteers who were recruited through email listservs and announcements at the University of California San Diego through IRB protocol #150846.

PROJECT DESIGN AND METHODS

The experimental design for Objectives 1-4 is outlined in Figure 1.

Prior to all experiments, we sprayed desk surfaces in the room with a 10% bleach solution and allowed them to soak for 15 minutes before wiping off. Next we placed tiles on the bleached desk surfaces and sprayed them with 10% bleach, and allowed them to soak for 20 minutes before wiping off and rinsing with HPLC purified water.

Objective 1: The experiment was conducted with 13 participants touching 5 material types (plastic, glass, ceramic, metal, and wood; Figure 1A). Each material type was touched in sets of 5 replicates in consecutive order, each touched 10 times. Tiles were swabbed immediately thereafter.

Objective 2: On 3 separate days, plastic tiles were touched either 10 times, 20 times, or 30 times by 20 participants (Figure 1B). Tiles were swabbed immediately thereafter.

Objective 3: The experiment was conducted with 6 pairs of participants on both plastic and ceramic tiles since this material type has shown to be the most robust for recovering personalized skin microbes. The first person in the pair touched a tile 30 times followed by the second person

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touching it 20 times an hour later (Figure 1D). The following day, the order of participants was reversed. Tiles were swabbed immediately thereafter.

Objective 4: 12 participants each touched a plastic tile 20 times, rotating the tile between touches such that each quadrant of the rectangular tile were touched equally by each part of the hand (Figure 1C). One quadrant of the tile was sampled immediately following the 20 touches. A second quadrant was sampled after five minutes, while the third and fourth quadrants were sampled after 1 and 18 hours, respectively. A second set of experiments using just plastic tiles was conducted to increase the sample size and extend the persistence time to several days.

Objective 5: In collaboration with the Honolulu Medical Examiners office, samples were collected from deceased individuals and surrounding surfaces at five death scenes. Samples were then collected again after arrival at the morgue, and continuously at 6 hour intervals until an autopsy was performed.

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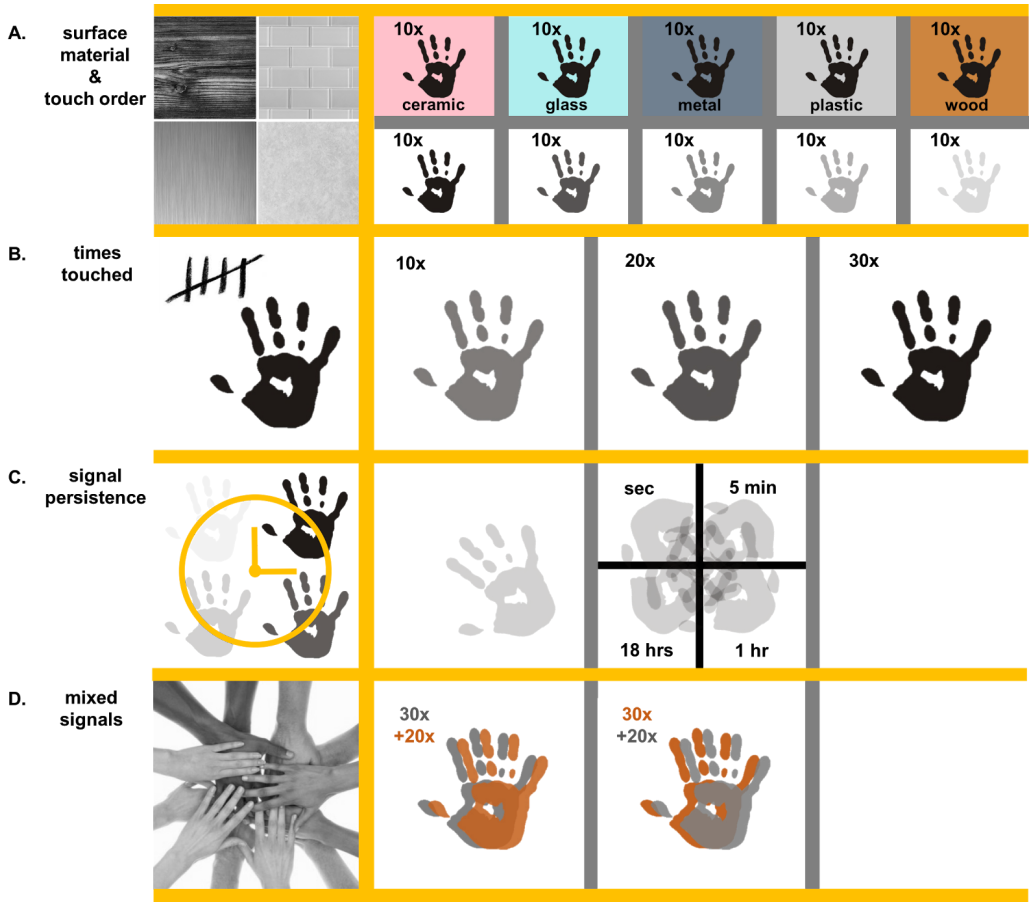

Figure 1. Schematic describing the four skin trace evidence experiments described in Phases 1-4. (A) Five replicates each of common surface materials, including ceramic, glass, metal, plastic and wood, were contacted with each participant's hand ten times. (B) The effect of the number of times each surface replicate was touched on the accuracy of linking a surface to a participant was tested. (C) The length of time personalized skin microbes persisted on a surface material was tested. (D) The ability to link personalized skin microbes to participants when a surface is touched by two people (e.g. victim and perpetrator) was assessed.

Sample collection and data generation: To characterize each participant's unique skin microbial signature, skin from each participant's hand was swabbed for five days leading up to, during, and/or following the experiment. BD BBL™ CultureSwab™ (Becton Dickinson, USA) sterile swabs were used to collect microbial DNA from participants' hands and surfaces. Swabs

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were immediately frozen at -20C until DNA extraction. Bacterial communities were characterized by extracting DNA, amplifying the 16S rRNA gene, and sequencing on an Illumina platform (MiSeq and HiSeq) following standard protocols (http://www.earthmicrobiome.org/protocols-and-standards/).

DATA ANALYSIS

- Sequence data were processed using tools in the QIIME (v.1.9.1.) software package. Processing included steps to assign sequences to samples, select out putative error-free sequences, and rarefy sequences to an even depth such that each sample is represented by the same number of sequences.
- We applied machine learning methods using a Random Forests classifier to match participants to the tiles they touched.
- To detect the pairs of participants in the multiple-person experiment (Objective 3), we used a Bayesian approach called SourceTracker (Knights et al. 2011) to estimate the relative contribution of the top two individuals identified as sources to the microbial profile of the sample.

FINDINGS

- Skin microbes detected on ceramic and plastic surfaces were most robustly and accurately linked to individuals (Figure 2).

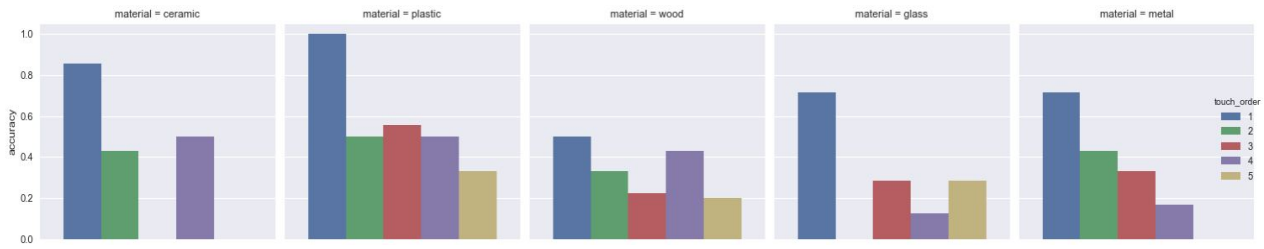

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Figure 2. Prediction accuracies for material types that were touched by participants in sets of 5, each touched 10 times. The first replicate for each material type was the most accurate (blue) for predicting the correct participant with plastic and ceramic demonstrating the highest overall accuracy.

- Unlike in our first experiment, our ability to recover enough sequence data from surfaces touched 10 times was poor in a second experiment in which we tested touch frequency; only surfaces for 3 out of the 20 participants yielded enough sequence data for analysis. However, 12 and 13 of the 20 surfaces touched 20 or 30 times respectively, successfully sequenced. We have determined that our ability to recover enough signal to correctly match the person with the tile they touched increases with 20 and 30 touches when compared to 10 touches (Figure 3).

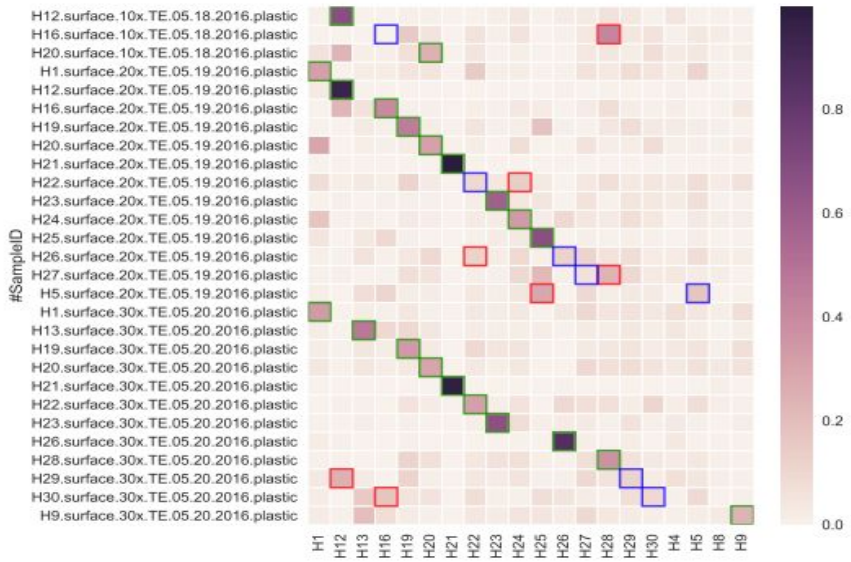

Figure 3. A heatmap showing the probability that a given individual contacted each plastic surface (dark = high probability). An outline in green indicates a correct match; if the prediction misclassifies, an outline in blue indicates the true donor and red indicates the misclassified prediction. The top 3 rows shows accuracies for the surfaces touched 10 times, the following 13 rows shows accuracies for the surfaces touched 20 times, and the last 12 shows

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accuracies for the surfaces touched 30 times. The prediction accuracy increases from 0.67 for 10x (2/3) and 0.69 for 20x (9/13), to 0.83 for 30x (10/12).

- We found that we could recover the skin microbial signatures of one or both people in many cases, and that some individuals leave a highly traceable signature against a background of someone else's signature while others do not (Figure 4).

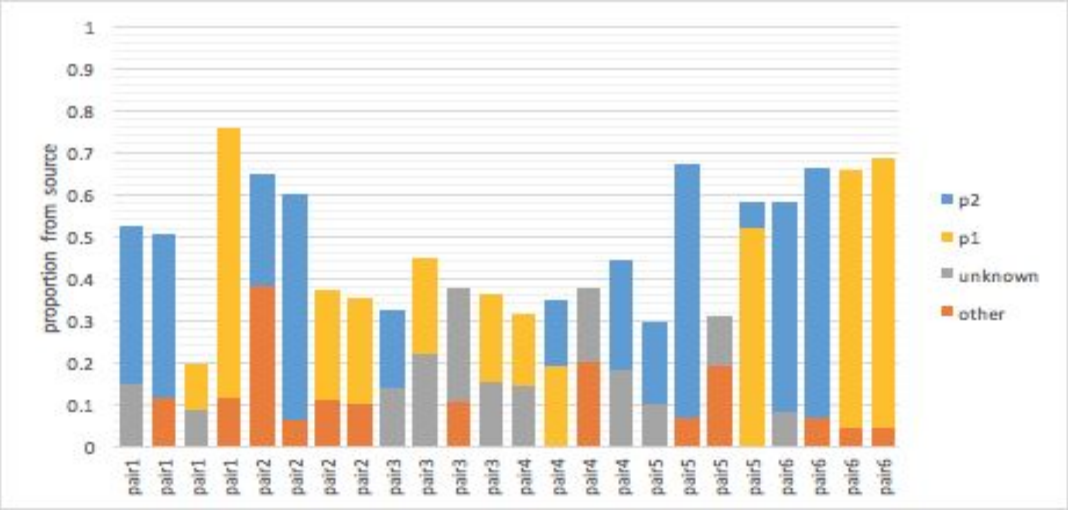

Figure 4. Sourcetracker analysis shows the relative contribution of the top two individuals identified as sources to the microbial profile of the sample. P1 and P2 refer to the first and second participant respectively. 'Other' indicates that a participant in the experiment other than the two people who contacted the tile was identified as contributing to the microbial profile of the sample. The four bars show alternating results for ceramic and plastic, respectively, with the first set of bars showing the instance when P1 touched the surface first and the second set of bars showing the instance when P2 touched the surface first. In most cases, the microbial signature of the person to last touch the surface (P2: blue bar)) was most robustly detected while in a few cases signatures from from both P1 and P2 were detected. However, one person's skin microbiome signature was sometimes "dominant", and would more accurately match the surface microbes regardless of the order in which the surface was touched.

- We found that a person's microbial signature can persist on a surface for at least one day.

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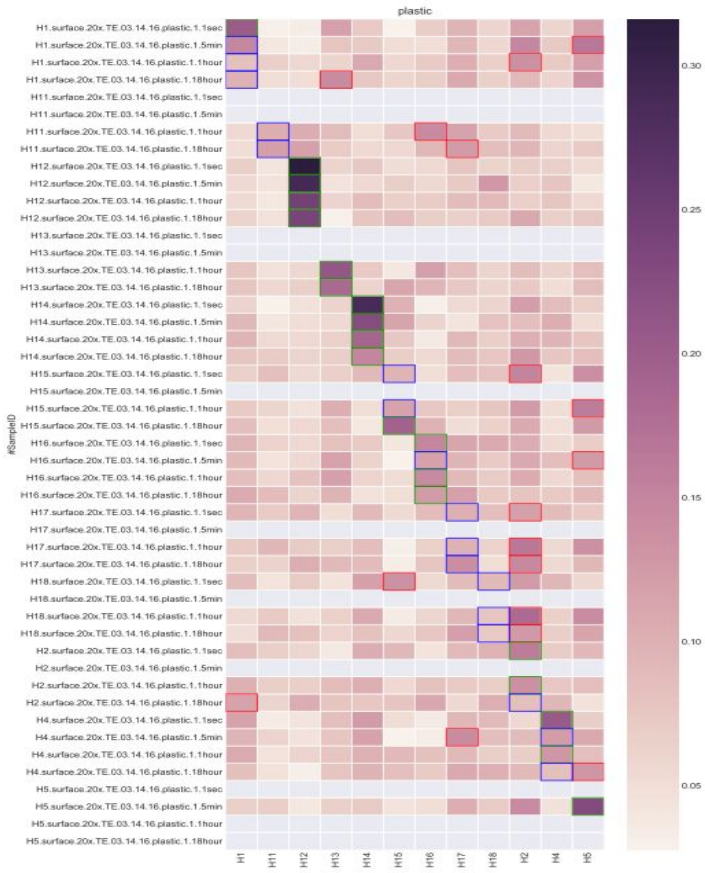

Figure 5. A heatmap showing the probability that a given individual contacted each plastic surface (dark = high probability), arranged by time elapsed. An outline in green indicates a correct match; if the prediction misclassifies, an outline in blue indicates the true donor and red indicates the misclassified prediction. The rows are ordered by individual and time (e.g. the first 4 rows are all surfaces touched by the same individual, sampled after 1 second, 5 minutes, 1 hour, and 18 hours).

- We found that the skin microbial communities on a deceased individual, whether sampled at earlier or later hours postmortem, still share unique/individualized microbial communities found on that individual's personal objects (Figure 6).

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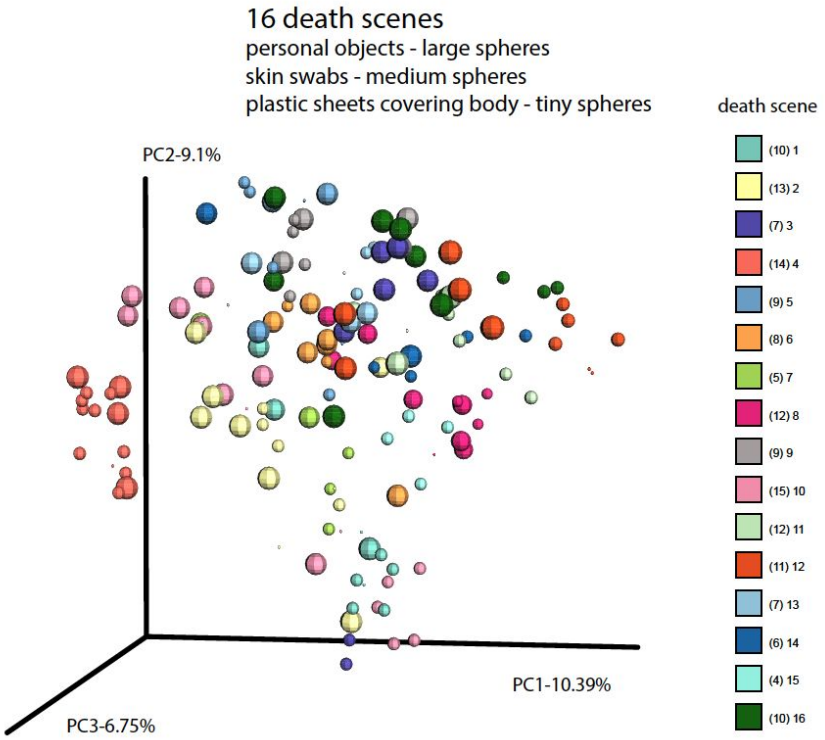

Figure 6. Samples from deceased individuals postmortem and their associated objects. Samples are colored by individual. The largest spheres are of the personal objects, medium-sized spheres are of the skin swabs across all time points (at scene of death, upon arrival at the morgue, and then at 6 hour intervals until an autopsy was performed), and the smallest spheres are of the plastic sheets covering the deceased body.

IMPLICATIONS FOR CRIMINAL JUSTICE POLICY AND PRACTICE IN THE UNITED STATES

By revealing new information about the transferability, stability, and individuality of human skin microbiomes, this forensic science research has increased our knowledge of physical evidence, providing basic information about the conditions under which criminal investigators can match an individual's skin microbial signature to objects or surfaces at crime scenes. This information is a crucial first step to further developing these technologies such that they can be used by

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general crime laboratories in the future. The current results are particularly exciting for criminal investigation because they demonstrate that skin microbes can also serve as individual evidence in a variety of scenarios. Most trace evidence, such as hairs and fibers, can only be used as class evidence as opposed to individual evidence. The recovery of a blonde hair shaft, for example, from a crime scene could be associated with anyone with blonde hair. Hair shafts very rarely possess characteristics that are unique to an individual. Fibers provide similar information. A blue cotton fiber recovered from a death scene could not be definitively linked to a blue cotton sweatshirt owned by a suspect, for example. Therefore, using skin microbes to identify individuals significantly expands the scope and power of trace evidence analysis. Our results also reveal two candidate surface types, plastic and ceramic, to focus technology development for recovering personalized skin microbial signatures as trace evidence at crime scenes.

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