DATA NOTE

REVISED Flow cytometry dataset for cells collected from touched surfaces [version 2; peer review: 2 approved]

Previously titled: Flow cytometry analysis of epithelial cell populations from touch samples using the BD Influx flow cytometry platform

Ye Jin Kwon, Cristina E. Stanciu, M. Katherine Philpott, Christopher J. Ehrhardt

Department of Forensic Science, Virginia Commonwealth University, Richmond, USA

V2 First published: 23 Mar 2016, 5:390
<https://doi.org/10.12688/f1000research.8338.1>
Latest published: 07 Oct 2016, 5:390
<https://doi.org/10.12688/f1000research.8338.2>

Abstract

'Touch' or trace cell mixtures submitted as evidence are a significant problem for forensic laboratories as they can render resulting genetic profiles difficult or even impossible to interpret. Optical signatures that distinguish epidermal cell populations from different contributors could facilitate the physical separation of mixture components prior to genetic analysis, and potentially the downstream production of single source profiles and/or simplified mixtures. This dataset comprises the results from antibody hybridization surveys using Human Leukocyte Antigen (HLA) and Cytokeratin (CK) probes, as well as surveys of optical properties of deposited cells, including forward scatter (FSC), side scatter (SSC), and fluorescence emissions in the Allophycocyanin (APC) channel. All analyses were performed on "touch" samples deposited by several different contributors on multiple days to assess inter- and intra-contributor variability.

Keywords

forensic science , flow cytometry , epithelial cell , touch mixtures

This article is included in the **Data: Use and Reuse** collection.

Open Peer Review

Approval Status

	1	2
version 2		
(revision)
07 Oct 2016	view	view
version 1
23 Mar 2016	view	view

1. **Dieter Deforce** , Ghent University, Ghent, Belgium

2. **Peter K Rogan** , Schulich School of Medicine and Dentistry, University of Western Ontario, London, Canada

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Christopher J. Ehrhardt (cehrhardt@vcu.edu)

Competing interests: No competing interests were disclosed.

Grant information: This project was funded by the National Institute of Justice Award number 2013-DN-BX-K033 (PI: Ehrhardt). Flow cytometry analyses were performed at the University of Virginia Flow Cytometry Facility which is supported through the University of Virginia Cancer Center National Cancer Institute P30-CA044579-23 Center Grant.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2016 Kwon YJ *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

How to cite this article: Kwon YJ, Stanciu CE, Philpott MK and Ehrhardt CJ. **Flow cytometry dataset for cells collected from touched surfaces [version 2; peer review: 2 approved]** F1000Research 2016, 5:390 <https://doi.org/10.12688/f1000research.8338.2>

First published: 23 Mar 2016, 5:390 <https://doi.org/10.12688/f1000research.8338.1>

REVISED Amendments from Version 1

In the revised manuscript we have expanded the dataset to include more contributors as well as aged samples. Additionally, we have included flow cytometry data from antibody hybridization experiments on touch samples involving Human Leukocyte Antigen and Cytokeratin Probes.

See referee reports

Introduction

Flow cytometry has proven a viable approach for differentiating cell populations in many types of uncompromised (i.e. non-degraded) forensic mixture sample (Dean *et al.*, 2015; Schoell *et al.*, 1999; Verdon *et al.*, 2015). However, application to ‘touch’ or trace epithelial cell mixtures remains a challenge since many cell surface features are lost or obscured during the process of keratinocyte differentiation, leaving few biochemical or structural features in shed corneocytes that vary between individual contributors. Preliminary research has identified specific optical characteristics—namely, red autofluorescence and forward scatter (FSC) and side scatter (SSC) profiles—that may vary between touch samples deposited by different contributors and collected immediately after deposition (Stanciu *et al.*, 2016). For this dataset, we built on these studies by examining optical properties such as these on an additional two flow cytometry platforms (including different settings, such as channel voltages). We also investigated the capacity of touch epithelial cells to bind to two different classes of antibody probes: Human Leukocyte Antigen (HLA), which has been successfully utilized to separate uncompromised mixtures of blood and other bodily fluids (e.g. Dean *et al.*, 2015) and Cytokeratin (CK), which is known to be a dominant component of epidermal cells. This dataset includes samples that were collected and analyzed immediately after deposition (i.e. ‘fresh samples’), as well as samples that were collected up to seven days after deposition.

Methods

Dataset 1. Touch epithelial samples

<http://dx.doi.org/10.5256/f1000research.8338.d137992>

Flow cytometry source data for all samples are provided in Flow Cytometry Standard (.fcs) format files. Source data files are organized into three different repositories labeled, “HLA Hybridization”, “Cytokeratin Hybridization”, and “Autofluorescence”. Flow cytometry data collected on aged touch samples is contained within the Autofluorescence data folder. File names are labeled with the anonymized sample ID number, date of analysis, and the instrument platform used. Source data files are also labeled by the specific probe used where applicable (e.g., AE1, HLA-A02, etc...) as well as by the voltage setting when different from those described in the above Methods.

Touch samples were collected from six volunteers using the following protocol which was approved by the VCU-IRB (#HM20000454_CR). Contributors either rubbed a sterile polypropylene conical tube (P/N 229421; Celltreat Scientific) for five minutes using their entire hand (i.e. palm and fingers), or held a

tube in their hands (no rubbing) for five minutes. Cells were then immediately collected from the surface with six sterile pre-wetted swabs (P/N 22037924; Fisher Scientific) followed by two dry swabs or were collected between 12 hours and seven days after deposition.

Cells were also deposited onto a wooden kitchen knife handle and the grip of a resin mold of a handgun by handling each substrate for five minutes. Several contributors donated samples on multiple days to assess intra-contributor variation of epithelial cell populations.

To elute the collected cells into solution, the swabs were manually stirred then vortexed for 15 seconds in 10 mL of ultrapure water (18.2 MΩ·cm).

Antibody hybridization

For antibody hybridization experiments, three milliliters of cell solution were centrifuged at 5,000×g for five minutes. The supernatant was decanted and the pellet was resuspended in 100 µl PBS buffer and 1 µL of Human Fc Receptor block (Cat# 130-059-901, Miltenyi Biotec) to increase the specificity of antibody binding before reaction with either HLA or CK probes. This suspension was allowed to incubate at room temperature for 10 minutes.

For HLA hybridizations, cells were then incubated with one of three different antibody probes for 30 minutes: mouse anti-human monoclonal antibody (mAb) HLA-ABC-FITC (Cat# 311403, BioLegend), HLA-A02-FITC, (Cat#343303, Biolegend), HLA-B7-FITC, (Cat#sc-53304, Santa Cruz Biotechnology). Cells incubated with anti-mouse IgG2a-FITC (Cat# 343303, BioLegend) for 30 minutes served as the isotype control for this set of hybridizations. Cells were then pelleted and washed once in 1x FACS buffer [PBS supplemented with 2% Fetal Bovine Serum (FBS, Cat# 100-106, Gemini BioProducts) and 10% Sodium Azide (Cat# S2002, Sigma-Aldrich)] and re-suspended in the same FACS buffer solution until flow cytometry analysis.

For CK hybridization experiments, cells were incubated with either cytokeratin probe ‘AE1’ (recognizes CKs 10, 14, 15, 16 and 19; Cat# 14-9001-80, Affymetrix eBioscience) or ‘AE3’ (recognizes CKs 1, 2, 3, 4, 5, 6, 7, 8), Cat# 14-900-80, Affymetrix eBioscience) for 30 minutes followed by reaction with a secondary antibody, anti-mouse IgG1-APC (Cat# 17-4015-80, Affymetrix eBioscience). We used anti-mouse IgG1-APC (Cat#17-4714-42, Affymetrix eBioscience) to create the isotype control for these experiments, incubating for 30 minutes. As before, cells were washed once and then resuspended in 1xFACS buffer prior to analysis.

Flow cytometry

Cell solutions—both those treated with antibody probe and those that were not—were passed through 100 µm filter mesh prior to flow cytometry. Flow cytometric analysis of cells was performed on three different platforms: BD FACSCanto™ II Analyzer, BD FACSARIA™ II High-Speed Cell Sorter, and BD Influx™ Cell Sorter (all from Becton Dickinson and Company, Franklin Lakes, NJ, USA). The FACSCanto and FACSARIA platforms were equipped with 488nm and 633nm lasers. The Influx cell sorter was equipped with 488 561, and 640nm lasers.

For HLA and CK studies, flow cytometry analysis was performed on the BD FACSCanto™ II Analyzer. Channel voltages were set as follows: Forward Scatter (FSC, 150V), Side Scatter (SSC, 200V), Alexa Fluor 488 (FITC, 335V), Phycoerythrin (PE, 233V; PE-Cy5, 300V; PE-Cy7, 400V), and Allophycocyanin (APC, 250V).

Autofluorescence studies of touch samples were performed on one of two BD FACSria™ II flow cytometers, or on the BD Influx Cell Sorter (additionally, analysis of unstained samples conducted on the FACSCanto, at the settings described above, are the equivalent of autofluorescence studies). Channel voltages for the FACSria were set as follows: FSC, 200V; SSC, 475V; and APC, 400V. Channel voltages for the BD Influx were set to the following: FSC, 17.5V; SSC, 16V; and APC, 74.6V.

Dataset content

Flow cytometry source data for all samples are provided in Flow Cytometry Standard (.fcs) format files. Source data files are organized into three different repositories labeled, “HLA Hybridization”, “Cytokeratin Hybridization”, and “Autofluorescence”. Flow cytometry data collected on aged touch samples is contained within the Autofluorescence data folder. File names are labeled with the anonymized sample ID number, date of analysis, and the instrument platform used. Source data files are also labeled by the specific probe used where applicable (e.g., AE1,

HLA-A02, etc...) as well as by the voltage setting when different from those described in the above Methods.

Data availability

F1000Research: Dataset 1. Touch epithelial samples, [10.5256/f1000research.8338.d137992](https://doi.org/10.5256/f1000research.8338.d137992) (Kwon *et al.*, 2016).

Author contributions

CE conceived the study. CE, CS, and YK designed the experiments. CS and YK carried out the research. CE and KP prepared the first draft of the manuscript.

Competing interests

No competing interests were disclosed.

Grant information

This project was funded by the National Institute of Justice Award number 2013-DN-BX-K033 (PI: Ehrhardt). Flow cytometry analyses were performed at the University of Virginia Flow Cytometry Facility which is supported through the University of Virginia Cancer Center National Cancer Institute P30-CA044579-23 Center Grant.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

- Dean L, Kwon YJ, Philpott MK, *et al.*: **Separation of uncompromised whole blood mixtures for single source STR profiling using fluorescently-labeled human leukocyte antigen (HLA) probes and fluorescence activated cell sorting (FACS).** *Forensic Sci Int Genet.* 2015; **17**: 8–16.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kwon YJ, Stanciu CE, Philpott MK, *et al.*: **Dataset 1 in: Flow cytometry dataset for cells collected from touched surfaces.** *F1000Research.* 2016.
[Data Source](#)
- Schoell WM, Klitsch M, Mirhashemi R, *et al.*: **Separation of sperm and vaginal cells based on ploidy, MHC class I-, CD45-, and cytokeratin expression for**

- enhancement of DNA typing after sexual assault.** *Cytometry.* 1999; **36**(4): 319–323.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Stanciu CE, Philpott MK, Bustamante EE, *et al.*: **Analysis of red autofluorescence (650–670nm) in epidermal cell populations and its potential for distinguishing contributors to 'touch' biological samples [version 1; referees: awaiting peer review].** *F1000Res.* 2016; **5**: 180.
[Publisher Full Text](#)
- Verdon TJ, Mitchell RJ, Chen W, *et al.*: **FACS separation of non-compromised forensically relevant biological mixtures.** *Forensic Sci Int Genet.* 2015; **14**: 194–200.
[PubMed Abstract](#) | [Publisher Full Text](#)

Open Peer Review

Current Peer Review Status:

Version 2

Reviewer Report 28 December 2016

<https://doi.org/10.5256/f1000research.10476.r16849>

© 2016 Deforce D. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Dieter Deforce

Laboratory for Pharmaceutical Biotechnology, Ghent University, Ghent, Belgium

The authors have provided additional data making the dataset more relevant for a forensic setting.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 07 October 2016

<https://doi.org/10.5256/f1000research.10476.r16850>

© 2016 Rogan P. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peter K Rogan

Dept of Biochemistry, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, N6A 5C1, Canada

The authors have addressed my comments with additional data. The analysis of this data is ongoing, and not included in this paper.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 26 April 2016

<https://doi.org/10.5256/f1000research.8964.r13496>

© 2016 Rogan P. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peter K Rogan

Dept of Biochemistry, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, N6A 5C1, Canada

The authors describe a dataset used for flow cytometric analysis of sloughed epithelial cells from a set of 6 individuals. It is not at all clear why these data are different from those reported in their copublished research note (<http://f1000research.com/articles/5-180/v1>) which provides an analysis of these data. The anonymized names of the individuals in the two papers are the same and they are both in .fcs format. The Data Note is larger than the data in the Research Note, presumably because it contains technical replicates. The differences are trivial. In fact, the Research Note would have greater validity if the Data Note dataset was incorporated into this document and discussed there.

The second point that should be addressed is how the statistics of the fluorescence distributions (FS, SS) for different samples from the same individual or from different individuals can be compared given that the intensities vary, presumably as a result of the differences in yield from each sample. The reliability of using these histograms for making comparisons between replicates or individuals for forensic or any other applications could be suspect (eg. sample R12) due to noise, broad distributions or other factors. I suggest that the authors determine and provide minimum threshold criteria for analysis of a sample or comparison with other samples.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 29 Sep 2016

Christopher Ehrhardt, Virginia Commonwealth University, Richmond, USA

We agree that the differences between this manuscript and another publication currently in review at F1000 research could have been clearer. The cited manuscript (<http://f1000research.com/articles/5-180/v1>) was a preliminary survey of autofluorescence using one flow cytometry platform, the BD FACSCanto II Analyzer, whereas this study used two additional flow cytometry platforms, the BD FACS Aria II cell sorter and the BD Influx Cell Sorter; different channel voltages were applied on each of the three flow cytometers,

and the Influx is equipped with a different set of excitation lasers than the Aria and Canto. Additionally, for each manuscript a separate, non-overlapping set of touch samples was analyzed, although the reviewer is correct that some of the same donors contributed samples to both studies, albeit collected on different days. Given observed differences in fluorescence profiles for some contributors from one day to the next, sampling from the same contributor over multiple days adds to our understanding of this phenomenon.

To further distinguish this Data Note from previous publication we have significantly revised the Data Note to include a more comprehensive set of samples and experimental conditions, investigating both optical properties and antibody binding capacities of 'touch' epithelial cell populations. This includes flow cytometry data for autofluorescence as well as antibody hybridizations with HLA and Cytokeratin probes. As the reviewer suggests, we are also in the process of incorporating aspects of this dataset into other manuscripts that discuss the biochemical and forensic applications of flow cytometry analysis of touch samples (e.g., pre-print available at <http://biorxiv.org/content/early/2016/03/28/045948>).

We also agree with the reviewer's second point that there may be significant variability in the level of autofluorescence exhibited by touch samples. This in turn may be influenced by a variety of factors such as presence or absence of exogenous compounds, and/or intrinsic biological characteristics of the cells themselves. Understanding this variability, particularly for samples derived from the same contributor or for aged/degraded samples, is necessary to assess the potential utility of flow cytometry-based cell separation techniques such as FACS for downstream DNA profiling of separated cell populations derived from 'touch' biological mixtures.

We would note that although differences in cell yield change the *number* of cells (Y axis) fluorescing in the red portion of the spectrum at a given RFU value (X axis), it is nonetheless possible to develop a sense of how the average *intensity* of red autofluorescence exhibited by cells collected from different individuals, or from the same individual on different days, varies by comparing histograms, regardless of cell yield. We agree that an individual's total cellular contribution to a biological mixture will ultimately be an important factor in whether that individual's DNA profile can be successfully generated from a sorted mixture. However, any kind of minimum cell count threshold would be inextricably linked to the sensitivity/efficiency of downstream DNA typing methodologies used on sorted cell populations, and are beyond the scope of this Data Note.

Competing Interests: No competing interests were disclosed.

Reviewer Report 19 April 2016

<https://doi.org/10.5256/f1000research.8964.r13031>

© 2016 Deforce D. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Dieter Deforce

Laboratory for Pharmaceutical Biotechnology, Ghent University, Ghent, Belgium

The article suggests "touch samples" however the data set contains no data on forensic relevant touch samples. The six samples were from volunteers rubbing their entire hand. These are "fresh" cells and might not show the same flow characteristics as cells left behind on an object after a touch contact and having "aged" on the object. The dataset provided is as it is not really relevant to the forensic field.

In addition I think that 6 samples is maybe a too limited number for this kind of studies.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 29 Sep 2016

Christopher Ehrhardt, Virginia Commonwealth University, Richmond, USA

We agree with the reviewer that there is an important distinction between 'fresh' biological samples and ones that are aged and/or degraded since the latter is more likely to be encountered in forensic caseworking samples. Uncompromised samples can nonetheless provide an important foundation for future studies that explicitly examine the effects of aging/degradation on optical signatures identified by the initial study. We also note that there are forensic scenarios where fresh touch samples may be collected and analyzed for an investigation. For example, a firearm may be discarded by a suspect and immediately collected during pursuit by law enforcement.

However, to increase the forensic relevance of this dataset the new version of the manuscript includes samples that were aged between 12 hours and seven days before collection, as well as samples that were collected from forensically relevant substrates such as replica firearms and knife handles.

For the updated version of the manuscript we have also expanded the dataset to include 33 different contributors.

Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research