Document Title: 3D Morphology of Blood Stain Can Provide Critical Information Missed In Standard Bloodstain Analysis

Author(s): James Bird

Document Number: 308451

Date Received: January 2024

Award Number: 2020-DQ-BX-0006

Final Research Report

Federal award number: **2020-DQ-BX-0006**

Project title: **3D morphology of blood stain can provide critical information missed in standard bloodstain analysis.**

Principal Investigator: **James Bird**
Associate Professor in Mechanical Engineering

Contact information:

jbird@bu.edu
Dept of Mechanical Engineering, Boston University
110 Cummington Mall
Boston, MA 02215
617-358-6929

Award recipient organization:

Boston University
25 Buick Street
Boston, MA 02215

Project period (as it appears on the award document):

January 1, 2021 – December 31, 2022

Award amount: **$361,656**

Summary of the project:

Major goals and objectives:

Forensic scientists have long used blood stains to reconstruct crime scenes. In situations in which blood may be dripping from a wound, the size and shape of the stain is used to determine whether the wounded was walking or running; whereas in situations in which blood is spattered, the sizes of blood stains are often correlated with force of the trauma and the shape can provide insight into origin location, such as whether the victim was sitting or standing. To establish the origin of a blood stain, forensic analysts use a “stringing” technique. Specifically, using the width and length of the stain, an impact angle can be calculated. When an event leads to several nearby stains, the angles for these stains are each calculated with the assumption that where the tangential trajectories associated with these converge is the source of the splatter. Recent software packages have aimed to reduce the uncertainty in these estimates by accounting for parabolic paths and, if the size of the drop is known, drag from the air. To find these trajectories, information is needed on the drop size, impact angle, and impact velocity. However, it is less clear how to relate the final stain size and shape to these impact conditions. In particular, the role of gravity and substate wettability are typically neglected. Models typically assume that bloodstains are pinned to the substrate at largest point where they have spread; yet recent experiments have demonstrated that microscopic residues, such as superhydrophobic coatings and the oil resulting from fingerprints, can fundamentally change the shape of the final stain.

The goal of this project was to collect data on which common coatings and residues – often invisible to the eye – can dramatically alter the shape and size of bloodstains. A secondary goal was to investigate 3D morphological features to see if these contain the necessary information to detect the presence of the residues. The project was primarily experimental with a goal of generating extensive photographic data of human blood drop impact and drying under various conditions so that a fundamental understanding of the underlying fluid dynamics could be pursued.

Research questions:

The two major research questions that this project aimed to address are:

1. How does the advancing and receding contact angles affect the stain size, and are these angles are influenced by hematocrit?

2. Is there a difference between oblique and inclined impact on a blood stain pattern?

Research design, methods, analytical and data analysis techniques:

The approach consisted of systematic experiments on the impact and drying of drops of human blood. The drop size, impact speed, impact angle, target surface, hematocrit, and clotting rate were varied. From the collected images, the stain size, shape, color gradation, and crack propagation could be correlated with the varied substrate wettability, inclination, and blood drop properties and impact conditions.

Expected applicability of the research:

Our results demonstrate that bloodstains from drops on low adhesion and oily surfaces can be fundamentally different than those on cleaned surfaces. If the surface does not pin the blood contact line, we have found that it will appear that the stain impacted perpendicularly to the surface regardless of its true impact angle. Furthermore, we have found that if the drop can recoil or shrink after it has impacted the surface, it can be miscategorized as a non-drip drop. Thus our results have a direct impact on forensics.

Participants and other collaborating organizations

James Bird - Prof. Bird is an Associate Professor at Boston University in the Mechanical Engineering department. He acted as the Principal Investigator on this project and supervised the research.

Garam Lee - Ms. Lee is a doctoral student at Boston University and the lead student on this project. She created and tested different target surfaces and carried out the experiments with blood that obliquely impacts various surfaces. She has directed the undergraduates working with her on the project.

Alexandros Oratis - Mr. Oratis briefly participated in this project as a doctoral student at Boston University. He helped develop the experimental setup and update the institutional approvals to carry out the project with human blood.

Elijah Forstadt - Mr. Forstadt briefly participated in this project as a doctoral student at Boston University. He contributed to this project through numerical simulations and drop impact on oblique nonwetting surfaces.

John Morgan - Mr. Morgan briefly participated in this project as an undergraduate student. He explored the use of basic machine learning techniques to uncover patterns in the preliminary data. These included principal component analysis and neural networks.

Shulan Holmes-Farley - Ms. Holmes-Farley briefly participated in this project as an undergraduate student at Boston University. She worked on image processing and analysis with the blood stain data.

Yunting Yan - Mr. Yan briefly participated in this project as an undergraduate student at Boston University. He worked on image processing and analysis with the blood stain data, extending the work by Ms. Holmes-Farley after she graduated.

Armen Manucharyan - Mr. Manucharyan briefly participated in this project as an undergraduate student at Boston University. He is an undergraduate student at Boston University. He has worked on experiments of drop impact on inclined nonwetting surfaces.

Kenneth Martin - Det. Martin acted as a consultant on this project. He has participated in regular discussions and provided feedback on the initial results.

Daniel Attinger - Dr. Attinger acted as a consultant on this project. He has participated in regular discussions and provided feedback on the initial results.

Changes in approach from original design and reason for change

During our project our forensic consultants suggested a few changes in our approach based on the results that we were obtaining and their judgement on how to have the largest impact from our work. These recommendations and the subsequent changes were documented in our semi-annual progress reports and are summarized here.

First, our forensic consultants recommended that we reduce the blood drop size in the experiments. This change was helpful both because (1) the size that we originally planned is larger than is typically observed in forensic field work, and (2) smaller drop sizes will adhere to surfaces over a greater range of inclination angles, increasing the range of data that can be obtained. Switching to smaller drops required a different drop generating approach. Instead of dripping a single drop of blood, we needed to generate a moving jet that would spontaneously breakup into these smaller droplets (Figure 1). The size and speed of drops in this approach quite variable and required that we measure the size and speed of each drop upon impact using our high-speed camera.

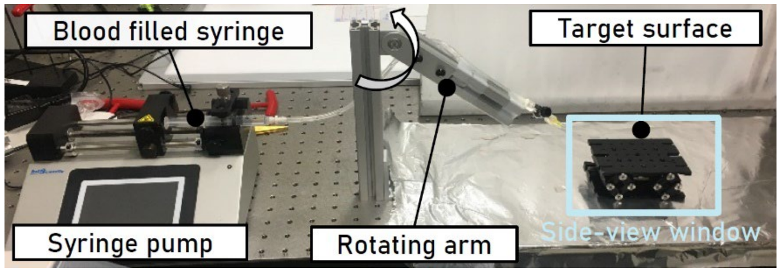

Figure 1. Oblique impact experimental setup.

A second recommendation was to modify the target substrates we were using. We initially chose surfaces with different residues, as we anticipated that clean, smooth surfaces would all behave identically. However, our initial data indicated that this assumption was not correct. It was therefore recommended that we include card-stock as one of the surfaces that we investigated and include surfaces with distinct equilibrium contact angles. Due to unexpected spatial variability in the contact angle on our glass slides, it was recommended that we carry out some of the experiments with acrylic and aluminum foil as the substrate. Additionally, to make the experiments more controllable and repeatable, we modified our approach to making oily surfaces by creating a liquid-infused surface so that the oil itself does not move on the substrate. Furthermore, when our forensic consultants alerted us that our work could be contextualized with the minimum drip-drop stain size experiments carried out by Gardner in 2006, we changed the smallest drop size in our vertical drip experiments to match those in Gardner's study. We also modified the impact materials to mirror those of Gardner (eg linoleum/vinyl and jeans) with and without commercially-available coatings (eg wax and water-repellent spray), as we believed that these changes would make our conclusions more impactful to practitioners. During the subsequent trials, we recognized the results will be clearer if focused on smooth surfaces for our experiments with varying hematocrit and removed the jean fabric from those experiments.

Final our forensic consultants recommended that we modify how we measure the length and width of a stain so that they are not influenced by tails that are observed at large impact angles. We recognize that the deviation between an ellipse centered on the front-edge of the stain and in the middle of the stain might itself shed some information on the characteristics of the impact and surface, and so used the difference in the two measurement approaches to define a tail length. It was suggested that we carry out some numerical simulations of the shape of liquid drops as they recoil on a non-wetting surface to provide insight into the shape of the stain. Due to resolution issues, these simulations were not particularly insightful, but with more effort might become more useful. Instead, we were able to correlate tail length experiments to provide insight into the drop impact conditions.

Outcomes

Activities/accomplishments:

The activities in this project can be categorized in four parts. The first involved characterizing the blood and the surfaces. The second involved blood drops released vertically onto a horizontal surface. The third involved blood drops released vertically onto an inclined surface. Finally, the fourth involved releasing drops obliquely on a horizontal and vertical surface.

Measurements were collected for the equilibrium contact angle, advancing and receding contact angles, surface tension, hematocrit, and the human blood viscosity. The contact angle was measured by slowly depositing blood onto the various surfaces. The advancing and receding contact angles were determined by finding the roll-off angle. The surface tension was calculated by the shape of a pendant drop, the hematocrit was measured visually after centrifuging the blood, and the viscosity was recorded using a viscometer (see Figure 2).

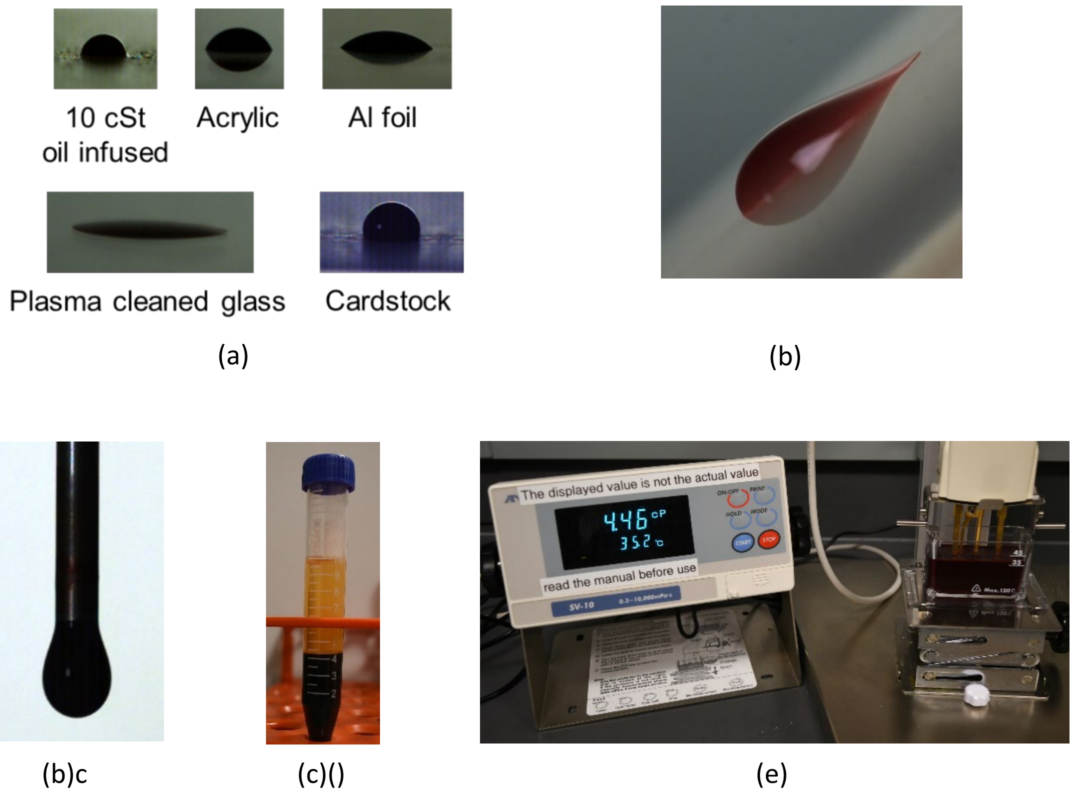

Figure 2. Liquid properties measurements: (a) Equilibrium contact angle (b) Advancing and Receding contact angles (c) Surface tension (d) Hematocrit (e) Viscosity.

The second activity investigated vertical impact on a horizontal surface. Various sized drops were released from a needle from different heights. The hematocrit was varied by centrifuging the blood, separating the plasma, and remixing it at different concentrations. In addition, the anti-coagulant was deactivated with sodium citrate in some experiments to investigate the effects of clotting.

The third class of experiments involved blood drops released vertically onto an inclined surface. For these experiments blood drops were released from a needle onto inclined target surfaces that were mounted onto angled jigs (Figure 3). The resulting stains were recorded perpendicular to the surface to ensure they remained in the focal plane (Figure 4).

For the last class of experiments, small drops impacted obliquely onto a vertical and on a horizontal target surface using the rotating arm setup illustrated in Figure 5. Four to five trails were taken each at 3 different velocities, at 4 different shooting angles, and on five different surface materials (glass, aluminum foil, oil infused surface, acrylic, and cardstock). High speed images of each trail were used to identify drops that remained isolated from others drops, and to measure their size, impact speed, and impact angle. Each drop was then linked to the image of its dried stain (Figure 5).

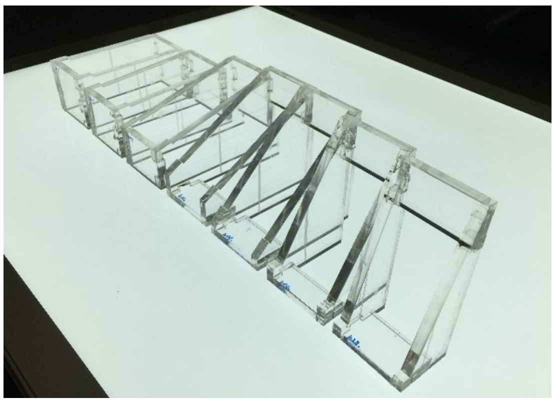

Figure 3. Angled jig for surface mounting.

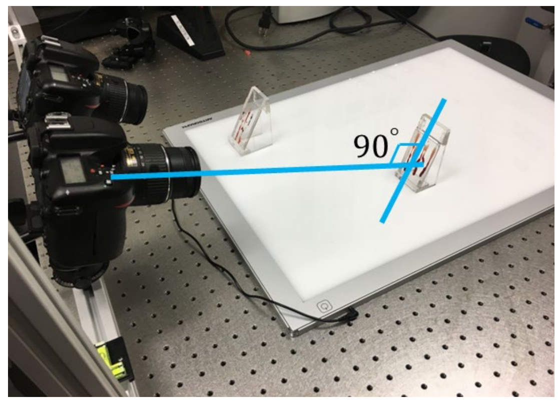

Figure 4. Stain image capturing setup perpendicular to the surface.

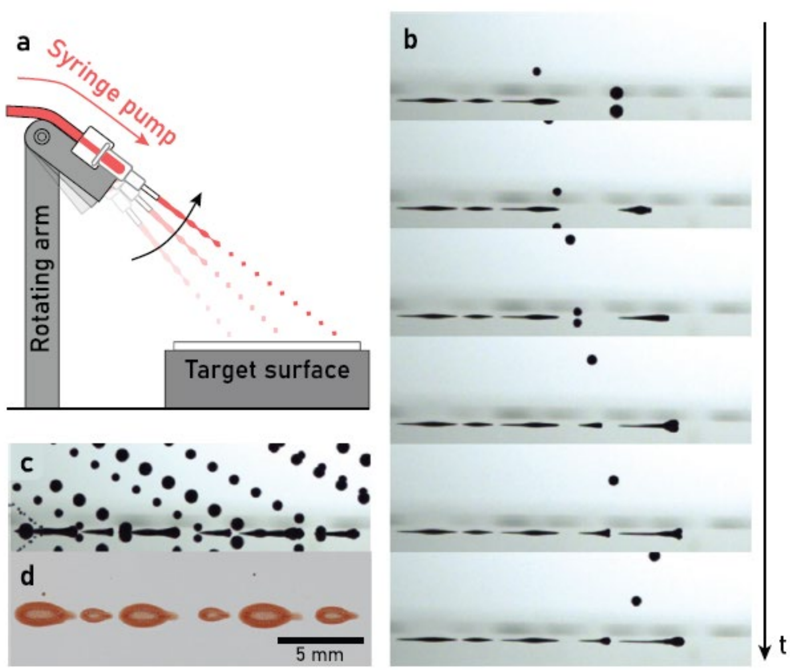

Figure 5. Oblique impact experimental setup.

Results and findings

Key results associated with the four above activities are outlined below. In addition to measuring the blood properties outlined above, we carried out some experiments to characterize the drying rate and clotting rate of the blood stains. It is anticipated that different-sized blood drops will dry at different rates. If the rate that the blood dried was spatially uniform, then the time for drying would depend on the stain surface area. However, past experiments of volatile liquids on smooth surfaces reveals that the evaporation rate at the contact line is significantly higher than the center of the stain. The consequence of this spatial variability leads to drying rates that scale with drop perimeter rather than area. Given the complexity of blood, it is not immediately obvious that this relationship would extend to bloodstains. Through systematic experiments, we demonstrated that it indeed does in the case that the blood contains anticoagulant. Furthermore, we found that when the anticoagulant is deactivated, the blood clots within 20 minutes. Therefore, we find that clotting appears to impact the larger drops that take longer than this time to dry. Figure 6 depicts how clotting influences the morphology of a blood stain at various hematocrits, illustrated through the color gradients and crack patterns.

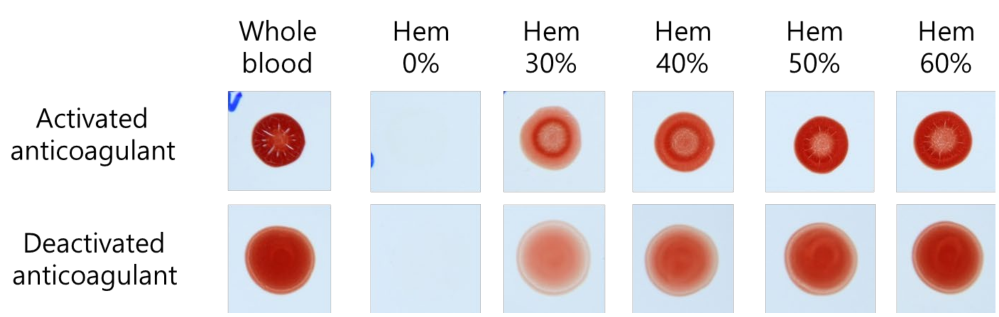

Figure 6. Deposition patterns of anticoagulant activated / deactivated blood with varying hematocrit.

Key results from our second class of experiments (vertical impact on horizontal surfaces) are illustrated in Figure 7. Blood drops of the same size, fall from the same needle height, and impact a wood surface. In one set of experiments the wood is bare, another it has been waxed, and in the third the surface is burnt so that it is covered with a thin layer of soot. On the bare wood, each drop spreads out to a maximum diameter, where it becomes pinned and so remains at this size as it dries. On the waxed wood, each drop spreads to the same maximum diameter, but is not pinned and retracts as it dries. Finally on the burnt wood, each drop spreads to the same maximum diameter, but air trapped in the soot creates superhydrophobic conditions that cause the drop to immediately recoil, leading to the smallest stain. Interestingly, the hematocrit does not appear to significantly alter these results.

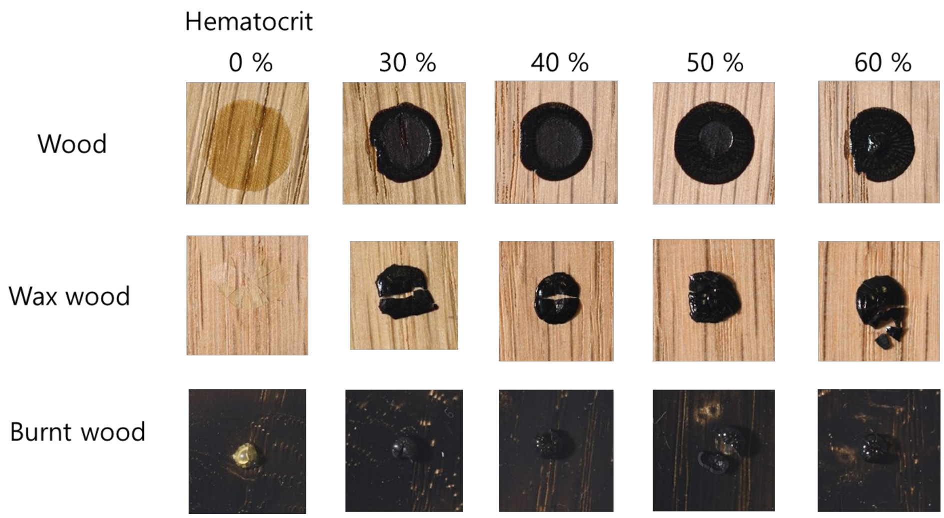

Figure 7. Bloodstains of varying hematocrit on surfaces with different coatings.

Key results from our third class of experiments (vertical impact on inclined surfaces) are illustrated in Figure 8. In this figure all the blood drops are released from 10 cm above the target. When the target is horizontal (zero degree inclination), the the stains are circular with a radial crack pattern. As the drops increase in size (which we accomplish by decreasing the gauge size of the needle from 34 G to 14 G), stain size increases. As the substrate inclination angle increases, the stain width decreases and stain length increases as expected; however the color gradients and crack patterns are quite remarkable and change considerably between the two surfaces. Note that at high inclination angles, the drop completely rolled off of the surface, leading us to move toward smaller drops generated from a breaking jet.

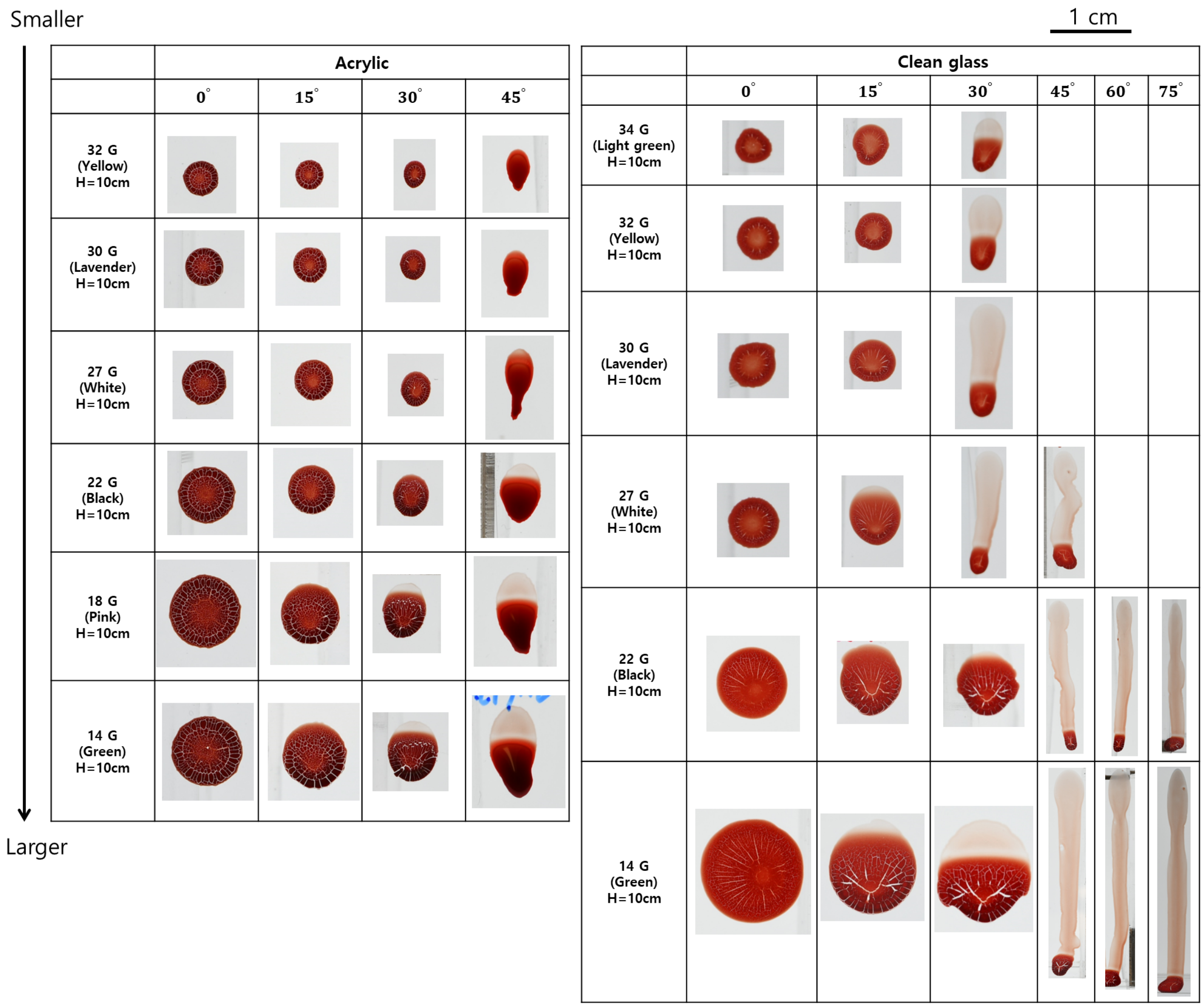

Figure 8. Bloodstain on Glass and Acrylic with different drop sizes.

Finally key results from our fourth class of experiments (oblique impact on horizontal and vertical surfaces from jet pinch-off) are illustrated in Figures 9 and 10. Blood drops generated in this set up are small enough that they adhere to a vertical surfaces when the surface is clean and bare, but still roll off the surface when it is non-wetting or oily. On a horizontal oily surface, the drop slides briefly before stoping and depositing a stain (Fig. 9). However, unlike on an acrylic surface where the aspect ratio of the length and width depends on the impact angle, there blood drop on the oily surface adopts a nearly circular shape regardless of impact angle.

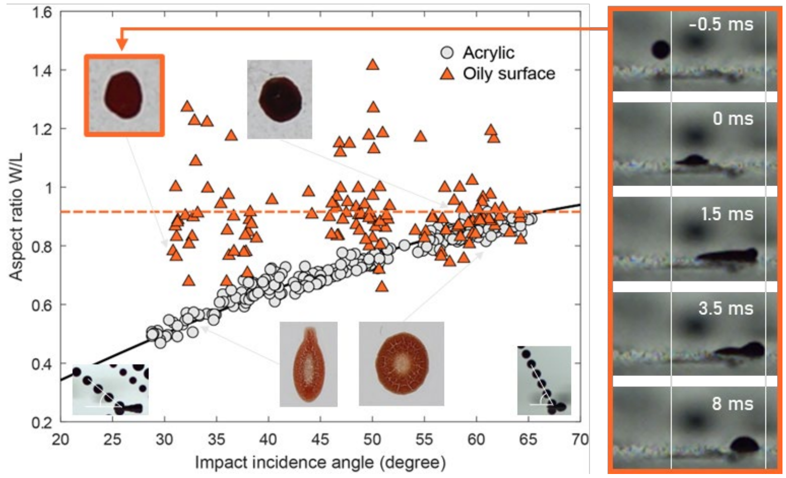

Figure 9. Impact angle comparison between the measurement value and the estimation by aspect ratio.

Representative images in Figure 9 show differences in the color and crack pattern for the stains on the acrylic and oily surfaces, illustrating that one can likely distinguish between pinned on unpinned stains based on the 3D morphology. For low incidence angles (which are equivalent a high surface inclination angle from Figure 8), there is a clear asymmetry in the stain that can not be attributed to gravity. Using the data from these experiments, we were able to show that this asymmetry depends not only on the impact angle, but also the drop size and impact velocity – providing qualitative data that can be used to calculate these parameters. High-speed images that we have taken reveal that the this tail develops during the final moments of impact, likely due to a combination of inertial, viscous, and capillary forces (Figure 10).

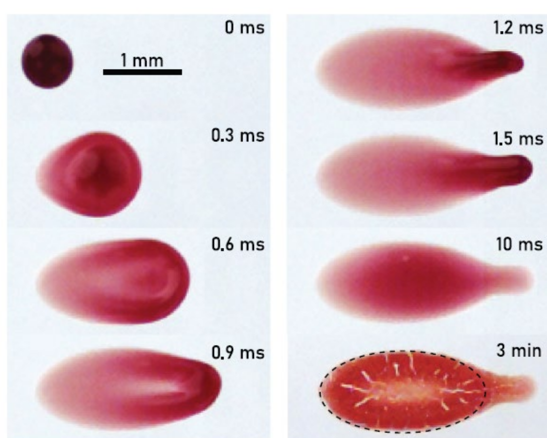

Figure 10. Tail formation on an obliquely impacting blood drop.

Limitations

Our results demonstrate that the stains from blood drops appear to depend strongly on whether the contact angle is pinned to the substrate. However, our results did not provide a clear indication of when depinning would occur. When depinning occurred, the final stains would typically be circular regardless of the impact angle – suggesting that it might be difficult to determine the source of the blood drops from the stain alone. When the blood drop remained pinned, we found that a tail developed for certain angles, although our analysis at the moment is restricted to small droplets on horizontal surfaces. Although we found correlation that related the tail length to the drop size, impact velocity, and impact angle (see published paper from 2023 listed on last page), the uncertainty in the exponents is significant. A key limitation is that we do not yet have a clear understanding the physics responsible for this effect, and therefore extreme caution should be taken if applying this correlation to stains taken outside of the range in which our study was conducted.

Artifacts

List of products (e.g., publications, conference papers, technologies, websites, databases), including locations of these products on the Internet or in other archives or databases

Lee, G., Attinger, D., Martin, K. F., Shiri, S., & Bird, J. C. (2023). Bloodstain tails: Asymmetry aids reconstruction of oblique impact. *Physics of Fluids*, *35*(11).

Lee, G., Attinger, D., Martin, K., Shiri, S., Bird, J.C. "Tail Formation in Obliquely Impacting Blood Drops" Presented at 76th Annual Meeting, American Physical Society, DFD, Washington, DC. Nov. 2023

Lee, G., Attinger, D., Martin, K., Shiri, S., Bird, J.C. "Revisiting the set value for the minimum drip stain size" Presented at NIJ forensic science R&D symposium. Online/Virtual Platform. Feb. 2023.

Lee, G., Verich, J., Greer, M., Bird, J.C. "Fat content and hematocrit: How do the constituents of milk and blood influence their final deposition patterns?" Presented at 75th Annual Meeting, American Physical Society, DFD, Indianapolis, IN. Nov. 2022

Lee, G., Attinger, D., Martin, K.F, Bird, J.C. "When bloodstain ellipticity depends on more than the impact incidence angle." Presented at NIJ forensic science R&D symposium. Online/Virtual Platform. Mar. 2022.

Lee, G., Shiri, S., Brasz, C.F., Bird, J.C. "The wicking of volatile drops on thin porous substrates." Presented at 74th Annual Meeting, American Physical Society, DFD, Phoenix, AZ. Nov. 2021.

Data sets generated (broad descriptions will suffice)

The data sets generated during this project have been deposited at https://dataverse.harvard.edu/privateurl.xhtml?token=82cde61f-85e1-4491-b277-4cb6fb1411b2 , which include the following contents.

- Blood liquid properties measurements results including density, viscosity, surface tension, and contact angle
- Highspeed videos capturing the impact dynamics and timelapse images providing drying processes under various impact blood drop impact scenarios
- Photographs documenting the experimental setups

Dissemination activities

Yang, Y., Lee, G., Bird, J.C. “Ellipticity dynamics of an obliquely impacting droplet.” 93rd New England Complex Fluid Seminar. Harvard University, Boston, MA. Dec. 2022

Lee, G., Attinger, D., Martin, K., Shiri, S., Bird, J.C. “Tail formation in obliquely impacting blood drops.” 95th New England Complex Fluid Seminar, Boston University, Boston, MA. Jun. 2023