BLOODSTAIN PATTERN ANALYSIS: INVESTIGATING THE EFFECTIVENESS OF SMALL (SUB 3MM) BLOODSTAINS IN AREA OF ORIGIN DETERMINATIONS

Brinda SALASKAR

A thesis submitted in fulfilment of the requirements for the degree of

Master of Forensic Science (Professional Practice)

in

The School of Veterinary and Life Sciences

Murdoch University

Project Supervisor: Mark Reynolds, Forensic Science Consultant, WA Police

Project Co-Supervisor: James Speers, Associate Professor in Forensic Science, Murdoch University

Semester 2, 2016

MURDOCH UNIVERSITY
PERTH, WESTERN AUSTRALIA

DECLARATION

I declare that this manuscript does not contain any material submitted previously for the award of any other degree or diploma at any university or other tertiary institution. Furthermore, to the best of my knowledge, it does not contain any material previously published or written by another individual, except where due references have been made in the text. Finally, I declare that all reported experimentations performed in this research were carried out by myself, except that any contribution by others, with whom I have worked is explicitly acknowledged.

Signed:

Dated:

Acknowledgements

The author would like to thank project partner Ryan Pugh for his assistance with selection and examination of impact patterns, WA Police Forensic Consultant Dr Mark Reynolds for his selection of large impact stains and his support and guidance on data interpretation and Associate Prof James Speers of Murdoch University's Forensic Science Department for his tireless dedication and advice on this project.

Table of Contents

Title Page .... **i**

Declaration .... **ii**

Acknowledgements .... **iii**

Table of Contents .... **iv**

List of Figures .... **v**

List of Tables .... **vii**

Part One

Literature Review **1**

1. Introduction 2
2. Origins of Bloodstain Pattern Analysis 3
3. Current Knowledge on Bloodstain Pattern Analysis 5
   - 3.1 Flight path of a Blood Droplet 5
     - 3.1.1 Forces Acting upon a Droplet in Flight 5
     - 3.1.2 Fluid Dynamics and Dimensionless Numbers 6
     - 3.1.3 Straight-line Trajectory vs. Ballistic Trajectory of Blood Droplet 8
   - 3.2 Bloodstain Selection 16
     - 3.2.1 Shape and Form of Bloodstain 16
     - 3.2.2 Impact Angle of Bloodstain 19
     - 3.2.3 Size of Bloodstain 22
4. Proposed Research 27
5. Glossary 28
6. References 29

Part Two

Project Manuscript **32**

Abstract 33

1. Introduction 34
2. Materials and Methods 38
3. Results 42
4. Discussion 51
5. Conclusion 55
6. References 56

List of Figures

Figure 1 Semi dimensionless plot to determine impact velocity of a falling droplet as a function of droplet diameter (d_o) and initial droplet velocity (v_o) for a vertical path of 1m (Attinger et al., 2013) 7

Figure 2 Diagrammatical representation of trigonometry associated with angle of impact calculations. The equation is a trigonometric function of a right angle triangle. (James et al., 2005) 9

Figure 3 Diagrammatical representation of the tangent method (image by Brinda Salaskar) 10

Figure 4 Visualisation of the manual string line method (image by David Spivey WA Police) 11

Figure 5 Calculation of angle of impact (α) using *Microsoft® Office Excel 2003 Auto Shape* program (image by Brinda Salaskar) 12

Figure 6 End View of AOO determination using BackTrack Program (Carter, 2001) 12

Figure 7 Impact velocity vector V shown in relation to its three components, V_x, V_y, and V_z and the virtual string. The three interlocking right triangles containing the three angles α, β and γ are also shown (Carter, 2001) 13

Figure 8 The positional instant of a droplet at multiple points along its ballistic path with straight line tangential indicators inserted (Reynolds, 2008) 14

Figure 9 Diagrammatical representation of angle of impact calculation theory. Sphere is projected onto a surface as an elliptical stain (James et al., 2005) 20

Figure 10 Graph showing how the error in calculating a bloodstain's angle of impact varies as a function of the bloodstain angle of impact itself (Pace, 2005) 21

Figure 11 Comparison between known and calculated angles of impact (α) for bloodstains ≤3.0mm long and >3.0mm and ≤6.0mm long that have fallen onto ceramic tiles at known impact angles. (Reynolds et al., 2009) 24

Figure 12 3D diagrammatical representation of AOO in relation to X, Y and Z co-ordinates (image by Brinda Salaskar) 35

Figure 13 Wooden apparatus and hammer used to create impact spatters (Evans, 2015)....39

Figure 14 Close up and technical photography of stain with scale using the Nikkon D5500 digital camera.. 40

Figure 15 Bloodstain used in the *Microsoft® Office Excel 2003 Auto Shape* program with axis, fitted ellipse and scale added (image by Brinda Salaskar) 41

Figure 16 Large, small and actual X co-ordinate comparisons for patterns 1-10................. 48

Figure 17 Large, small and actual Y co-ordinate comparisons for patterns 1-10................. 48

Figure 18 Large, small and actual Z co-ordinate comparisons for patterns 1-10................. 48

Figure 19 Average displacement of large bloodstains' calculated X, Y and Z co-ordinates from actual values for patterns 1-10 49

Figure 20 Average displacement of small bloodstains' X, Y and Z co-ordinates from actual values for patterns 1-10.. 50

List of Tables

Table 1 Significance of the physical regimes, shown as specific regions in Fig. 1. (Attinger et al., 2013)..7

Table 2 Increase in impact angle calculation error by the same amount of measurement error over the range of impact angles 25°-70° (Pace, 2005)...21

Table 3 *Microsoft® Office Excel 2003 Auto Shape* measurement data for large bloodstains l >3mm and small bloodstains l < 3mm **(IN BRACKETS AND BOLDED)** for area of origin determinations for impact patterns 1-10..44

Table 4 ANOVA statistical data for variances between small, large and actual X, Y and Z values for impact patterns 1-10...45

Table 5 Paired t-test data for variances between experimental (large and small) and actual X, Y and Z values for impact patterns 1-10..45

Page left intentionally blank

Part One

Literature Review

Investigating the Size, Shape, Formation and Ballistic Trajectory of Bloodstains in Bloodstain Pattern Analysis

1. Introduction

Bloodstain pattern analysis (BPA) has been used for centuries to help in the effective reconstruction of bloodletting events that occur at crime scenes (Attinger et al., 2013). The International Association of Bloodstain Pattern Analysts (2007) define these events to be as a result of, *"liquid blood being acted upon by physical forces causing bloodstain and bloodstain patterns to be deposited on various surfaces".*

The reconstruction of these events can help investigative bodies to gain a better understanding of critical information that can assist them in the evaluation of witness statements and crime participants' version of events (Peschel et al., 2011). Reynolds (2008) delves deeper into the complexities of the term 'reconstruction' to describe is as;

"The examination of the location, size, shape and distribution of bloodstains and bloodstain patterns in association with knowledge of the underpinning sciences (Mathematics, Physics, Chemistry and Biology) to provide information on an event, or sequence of events, that have resulted in the deposition of those bloodstains or bloodstain patterns; a scientific means by which physical, spatial and temporal components of a crime event can be inferred or confirmed."

Bloodstain pattern analysis and its many facets have become pivotal in determinations of bloodletting events due to the ability of the analyst to determine the area of origin (AOO) of each event. There are specific selection criteria when choosing bloodstains for this type of determination which will be discussed in this literature review.

2. Origins of Bloodstain Pattern Analysis

From its early days in 19th century Germany (Attinger et al., 2013), bloodstain pattern analysis (BPA) has strived to understand the "*how?*" part of the crime puzzle. Although informally used and researched for many years, the very first known systematic study of bloodstains was published in 1895 by Eduard Piotrowski from the University of Krakow titled, *'On the formation, form, direction and spreading of bloodstains after blunt trauma to the head'*. Through his research of blunt force trauma on live rabbits Piotrowski was able to visualise and study the patterns of bloodstains created, and postulated that the condition for the appearance of bloodstains was the presence of an existing blood source (Piotrowski, 1895), a finding still widely accepted to this day.

In 1954, P.L. Kirk referenced BPA in his expert testimony of a murder trial committed in Ohio, USA *(State of Ohio vs. Samuel Sheppard)*. Kirk showed the position of the assailant and the victim through his reconstruction and revealed that the attacker struck the victim with his left hand, which was significant as Sheppard was right-handed. Kirk later went on to develop a research project based on the case and although it remains unsolved to this day, the case marked a pivotal moment in BPA history as expert witness testimony had never been given on the discipline before this time.

Other pioneers of BPA such as Dr. Victor Balthazard and Dr. Herbert Leon MacDonell were the first to publish articles on angles of impact (Balthazard et al., 1939) and flight characteristics of human blood (Macdonell, 1971) respectively. Balthazard presented an article *'Etude des Gouttes de Sang Projete'* to the XXII Congress of Forensic Medicine outlining the elements of a bloodstain that gave indications to its origin and proposed the idea that a trigonometric sine relationship existed between the length and width of a bloodstain ellipse. He proposed that accurate measurements of the ellipse would lead to the determination of impact angle *(α)*. Macdonell was the first to demonstrate repeatability of the discipline as his work and results were remarkably similar to those done by Balthazard. In 1973, Macdonell founded the Bloodstain Evidence Institute and developed a basic bloodstain analysis course for law enforcement personnel. He eventually went on to attend the first Advanced Bloodstain Institute, and along with other attendees founded the International Association of Bloodstain Pattern Analysts (IABPA) in 1983.

In 1988 Dr. W. Eckert released a comprehensive book on the '*Interpretation of Bloodstain Evidence at Crime Scenes'* and five years later Dr. Chris Price and Adrian Emes were the first to release a video, 'Blood in slow motion', illustrating how blood behaved under various conditions using 4,000 frames per second recording footage.

In 2002, the Scientific Working Group on Bloodstain Pattern Analysis (SWGSTAIN) was formed as a means to create 'best practises' among public and private sectors on the disciple internationally. SWGSTAIN promoted and enhanced the development of quality forensic BPA practices through the collaborative efforts of government forensic laboratories, law enforcement, private industry and academia.

3. Current Knowledge on Bloodstain Pattern Analysis

There exists a large knowledge base on BPA, ranging from its uses in crime scenes, to its evidentiary value in court and its impact on legal proceedings. Among the array of bloodstain patterns that can be distinguished, an impact spatter is of particular evidentiary value and forensic interest. Impact spatter is described by the Scientific Working Group on Bloodstain Pattern Analysis (SWGSTAIN) as*, "a bloodstain pattern resulting from an object striking liquid blood"*. The radiating pattern of individual droplets that results from this can help investigators in spatial, temporal and contextual reconstruction of the events resulting in bloodshed. In order to use impact spatter patterns in such cases it is vital to understand the characteristics of a blood drop and the forces acting upon its flight path.

3.1 Flight path of a Blood Droplet

3.1.1 Forces Acting upon a Droplet in Flight

Kabaliuk et al., (2014) investigated the forces acting upon fluid (blood) in motion in his work on droplet deformation and breakup, referencing the effects of air currents and wind on spatter drop trajectories. The study found that when a blood drop was following a ballistic trajectory it was influenced by gravitational, inertial and aerodynamic drag forces and depending on the mechanism of formation, the fluid drop may be non-spherical and experience shape oscillations under the influence of surface tension (Kabaliuk et al., 2014). These forces are the source of constant critique and research in the forensic world due to the impact they have on AOO determinations. The article also introduced a single model found to describe drop behaviour accurately in passive, cast off and impact scenarios. The model was a numerical code for accurate modelling of blood drop flight path that incorporated gravitational and aerodynamic drag forces as well as in-flight drop deformation, oscillations and possible secondary break-up scenarios. Although this article included detailed description on the mechanisms of spatter drop generation and the uses of the proposed numerical model, it did not sufficiently detail the influences and impacts of gravitational and drag forces on a blood drop trajectory.

3.1.2 Fluid Dynamics and Dimensionless Numbers

Attinger et al., (2013) produced a more comprehensive analysis on these forces, comparing bloodstain pattern analysis to fluid dynamics. The comparative review highlighted the relationships between the disciplines of bloodstain pattern analysis in forensics and fluid dynamics in the physical sciences. The article made some very valid connections between the two disciplines and revolved around the physical forces driving the motion of blood as a fluid; the generation of drops; their flight in the air; their impact on solid or liquid surfaces and the production of stains. It described interactions between forensics and physics with their joint use of dimensionless numbers, suggesting that proper use of such numbers in the forensic field resulted in simpler analyses valid for a wider range of experimental conditions thus allowing experimental outcomes to be inferred for cases that *had not* or *could not* been tested explicitly. Dimensionless numbers such as the Weber number and Reynolds number are often used in forensic science in a semi-dimensionless plot (Figure 1) to determine the impact velocity of a falling droplet.

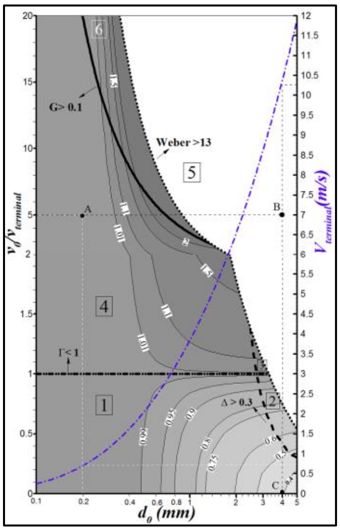

Figure 1 Semi dimensionless plot to determine impact velocity of a falling droplet as a function of droplet diameter (d_o) and initial droplet velocity (v_o) for a vertical path of 1m (Attinger et al., 2013)

The dimensionless numbers allow for the presentation of a wide range of physical regimes in the same plot and answer fundamentally significant questions in BPA studies (Table 1).

Table 1 Significance of the physical regimes, shown as specific regions in Fig. 1. (Attinger et al., 2013)

Physical Regime	Physical significance on the trajectory of a drop
1	Significant acceleration of droplet due to gravity forces, drag forces are significant
2	Significant acceleration of droplet due to gravity, drag forces are insignificant
3	Minor deceleration of droplet due to drag, gravity negligible with respect to drag, but gravity significant with respect to initial kinetic energy
4	Drag forces significantly decelerate the drop; gravity negligible with respect to drag, but gravity significant with respect to initial kinetic energy
5	High probability that drop breaks up into smaller drops during flight, due to shear breakup
6	Major deceleration due to drag; gravity negligible with respect to initial kinetic energy

Attinger et al., (2013) also mentioned one of the most debated topics in BPA: the use of straight-line trajectories in AOO calculations. It has been discussed in many articles dating back to those by Piotrowski (1895) and Balthazard et al., (1939) that blood follows a 'bent' or 'ballistic' trajectory and that reconstruction methods based on a straight-line trajectory carry with it uncertainties and errors. The article goes on to briefly mention the errors associated with these methods and the assumptions used in the calculations. Although the article gave an exhaustive review of the two disciplines in great detail and their overlapping interests, it did not spend enough time fulfilling the initial aim of identifying potential inter-community collaborations or suggest the direction of future endeavours that would help the two disciplines work most effectively together.

3.1.3 Straight-line Trajectory vs. Ballistic Trajectory of Blood Droplet

One of the most researched topics in BPA is the error associated with AOO calculation models that assume blood droplets follow a straight-line trajectory. From its myriad of uses, one critical function of BPA at a crime scene is its effectiveness in determining the AOO of a bloodletting event. AOO determinations are of particular forensic interest as they define the three dimensional location where a source of blood originated. The width and length of a blood droplet enables determination of the impact angle by using the equation $Angle\ of\ Impact\ (\alpha) = arcsine\left(\frac{width}{length}\right)$ (Figure 2). Previously, the impact angle *(α)* and glancing angle (γ) were used to compute the direction of the droplets chosen for the determination at the instant before impact in directional analysis method introduced by Carter (2001). These droplets were then assigned virtual strings and subsequently by measuring the x, y and z co-ordinates of the bloodstain, a point of origin could be estimated at the intersection of these strings.

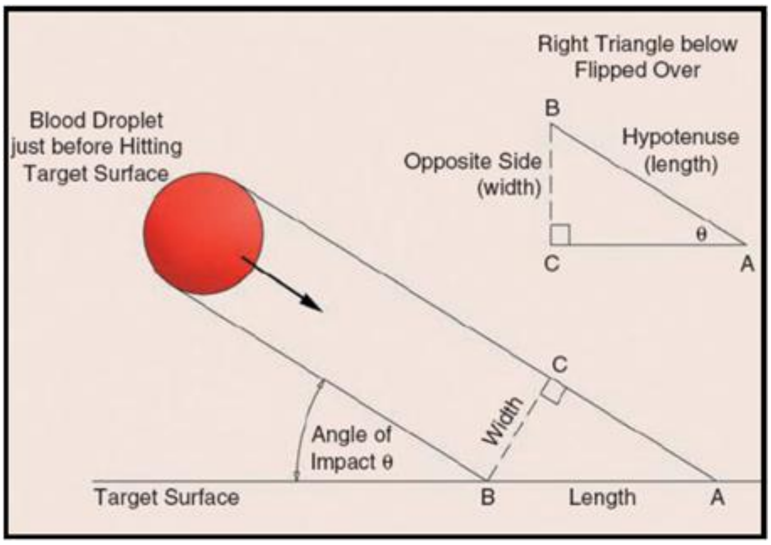

Figure 2 Diagrammatical representation of trigonometry associated with angle of impact calculations. The equation is a trigonometric function of a right angle triangle. (James et al., 2005)

All current AOO determination methods are based on Euclidean geometry and straight-line blood droplet flight path predictions. In other words, the methods are predicated on the assumption that the path of a blood drop away from the blood source follows a straight-line trajectory, thus eliminating the effects of gravity and drag. As such, the calculated Z value (height of blood source) will always be higher than the actual Z value, providing an upper blood source Z value limit. Furthermore, there is a common underestimation of calculated impact angle *(α)* values due to an overestimation of ellipse length relative to width in the initial phase of calculation. This results in a calculated X value (distance from impact wall) being closer than the actual blood source X value (Reynolds et al., 2009) and will be discussed further on in this review.

The three most often used methods for AOO determinations are; 1) Tangent Method, 2) Manual Stringline Method and 3) Computer Assisted Determination. The tangent method relies upon the trigonometric relationship of a right-angle triangle: Tan α= opposite/adjacent. In this method, the axis of each stain is determined and a straight line is drawn through the middle of it. The area of convergence (AOC) is then determined at the intersection of the lines created by each of the stains. The angle of impact *(I°)* is calculated using the equation $I° = \sin^{-1}(width/length)$ and is substituted into the equation as the "α" value. A measurement is made from the leading edge of each stain to the AOC and is substituted into the equation as the "*adjacent*" value. The "*opposite*" value is calculated using a rearrangement of the tangent equation and is also known as the height of the blood source. The tangent method is diagrammatically shown in Figure 3.

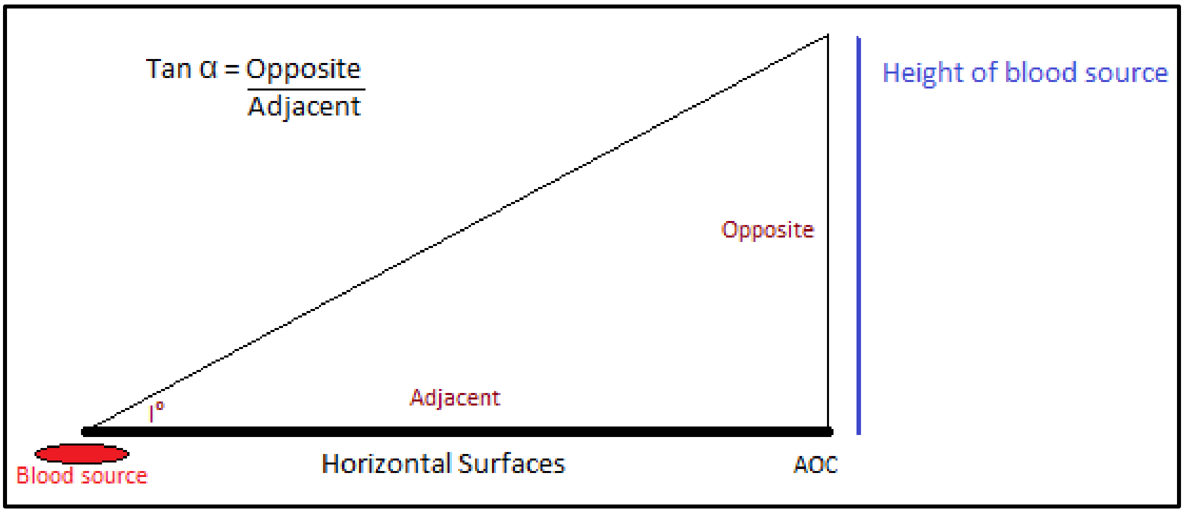

Figure 3 Diagrammatic representation of the tangent method (image by Brinda Salaskar)

The manual stringing method uses the same premise by calculating the angle of impact *(α)* but then utilises a protractor to mark the second point of attachment for the stain using strings. Each stain has a second point attached and the area at which they converge visually is determined to be the AOC. The height and distance can then be manually distinguished using a ruler (Figure 4).

Figure 4 Visualisation of the manual string line method (image by David Spivey WA Police)

The computer assisted method of determination use accurate stain measurements based on the mathematical properties of an ellipse and generate virtual strings that are perfectly shaped and located. Figure 5 shows the calculation of impact angle using the *Microsoft® Office Excel 2003 Auto Shape* program and Figure 6 is the end view of an AOO determination using the *BackTrack*™ program.

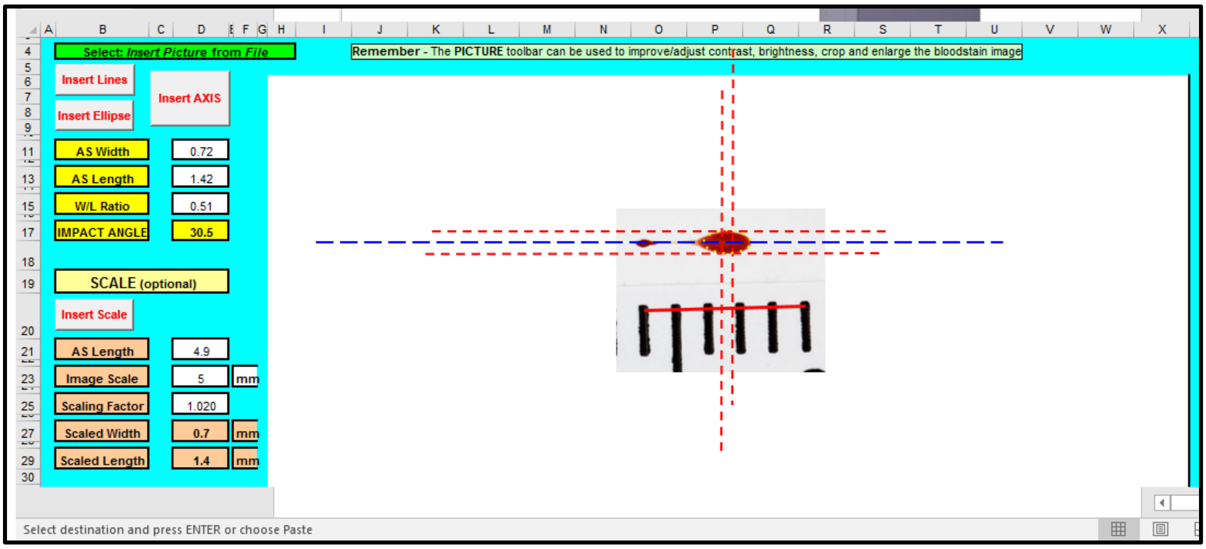

Figure 5 Calculation of angle of impact (α) using *Microsoft® Office Excel 2003 Auto Shape* program (image by Brinda Salaskar)

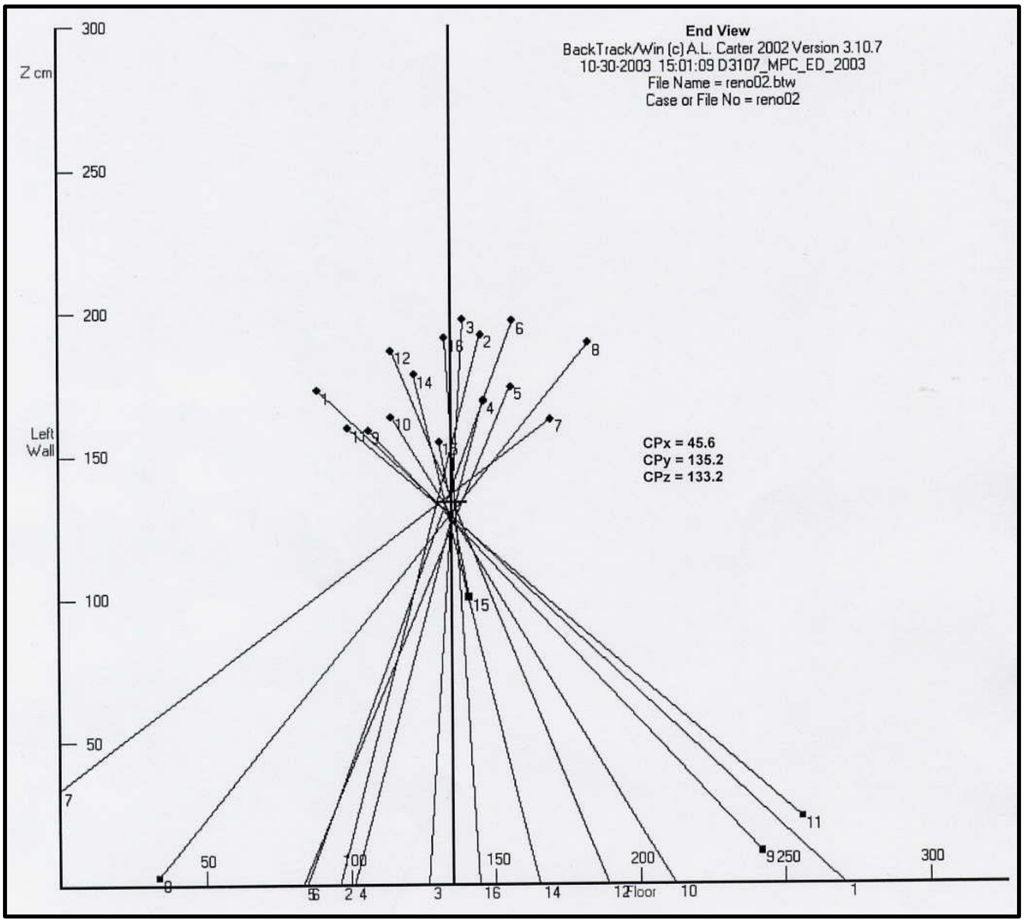

Figure 6 End View of AOO determination using BackTrack Program (Carter, 2001)

Carter (2001), was one of the first to use directional analysis to calculate the AOO of a bloodstain. He used a model to theoretically and practically predict the AOO of a bloodstain (Figure 7).

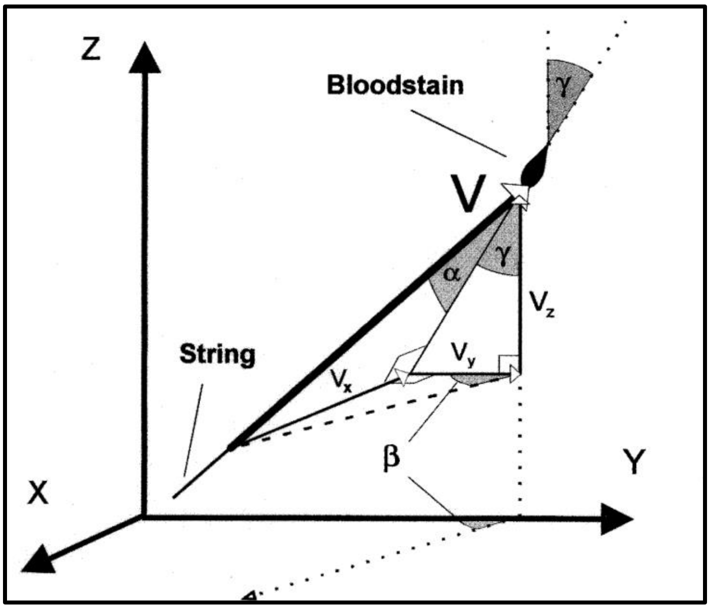

Figure 7 Impact velocity vector V shown in relation to its three components, V_x, V_y, and V_z and the virtual string. The three interlocking right triangles containing the three angles α, β and γ are also shown (Carter, 2001)

As shown in Figure 7 the X, Y and Z co-ordinate system assigns three planar reference co-ordinates for area of origin determinations. The X-value relates to the parallel vertical planar distance away from the spatter bearing surface. The Y value assigns a vertical planar distance from a referenced point (usually a 90° wall intersection) at some point along the spatter bearing surface and the Z value is an indication of the horizontal plane height above a referenced horizontal surface (usually a floor) (Reynolds, 2008).

In his article, 'The directional analysis of bloodstain patterns theory and experimental validation' (2001), Carter assessed two commercially available computer programs:

HemoSpat© and *BackTrack*™. This study along with future studies confirmed previously held views on the programs; the z co-ordinate (height) of the blood source was almost always overestimated due to the contributions of gravity and air resistance being neglected on drop flight path (Carter et al., 2005, Carter et al., 2006, de Bruin et al., 2011). Furthermore, de Bruin et al., (2011) found that bloodstains located higher on the wall or more than 50cm away from the point of origin produced the largest source of error as gravity was a more influential factor on these flight paths.

In another study on flight path it was stated that, “due to the unknown curvatures of the individual flight path, unknown systematic errors in the horizontal value of the location of the blood source may occur” (Buck et al., 2011). This was in agreement with the thesis *‘Bloodstain size, shape and formation-implications for the bloodstain pattern analyst’* by Reynolds (2008). Figure 8 demonstrates the positional instant of a droplet at multiple points along its ballistic path with the directional components of the gravitational (blue arrows) and air resistance (pink arrows) force vectors indicated. It also shows straight-line tangential indicators (black lines) from the same position on the droplet as it travels along its flight path.

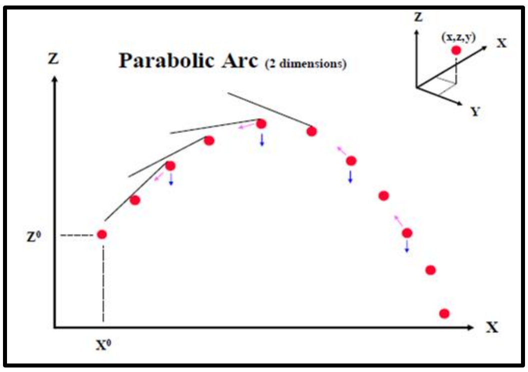

Figure 8 The positional instant of a droplet at multiple points along its ballistic path with straight line tangential indicators inserted (Reynolds, 2008)

Buck et al., (2011) aimed to evaluate the ballistic analyses used to approximate the trajectories of blood drops for AOO determinations. It highlighted the main problem associated with cases where there was a longer distance between the AOO and impact surface; a deviation of the vertical component (height) from the expected centre of origin due to the curved trajectory of the drop. According to the study, due to this source of error, ballistic analysis of the bloodstain pattern in these cases was critical to confirm the bloodstains were from the determined blood source location. The study used photogrammetry and 3D scanning methods to document the bloodstains and then used computer-aided design (CAD) software to compute the length of the major and minor axes of the ellipses. It revealed that 3D documentation and the use of ballistic determination methods enhanced the bloodstain pattern analysis reconstruction and furthermore gave clues as to the number and position of the areas of origin, the number of blows, the positioning of the victim and the sequences of events.

Connolly et al., (2012) on the other hand, sought out to validate the use of straight line trajectories in their article on the effect of impact angle variations on AOO determinations. The research article hypothesised that impact angles of between 5° and 15° would have negligible influence on AOO estimations. Furthermore, it was hypothesised that the size of the area of origin would affect the influence of the angle of impact variations, thus validating the use of straight-line trajectories as a robust and reliable method. The study concluded that the size of the area of origin determined the level of accuracy necessary in angle of impact estimations, *i.e.* as the size of the true area of origin increased, larger errors were tolerated in the estimation. Additionally, the study also showed that an impact angle of ±5° could, in certain conditions, produce a significant difference between the true and estimated areas of origin however, this required all bloodstain angles to have errors of ≥5°and an area of origin ≤14cm. It also stated that large inaccuracies may occur in some impact angle calculations but did not result in a significant change in the area of origin estimate. It was suggested that the utilisation of the bloodstain selection model by Illes and Bouè (2011) was sufficient enough to enable the use of straight-line trajectories and generate a valid and reliable estimate of point of origin (Connolly et al., 2012).

3.2 Bloodstain Selection

Due to the potential source of error regarding straight-line trajectories mentioned above, there are specific characteristics of blood droplets sought out by BPA analysts in their AOO determinations that help minimise or reduce these effects of error and increase accuracy. Stain selection is the first of these factors as the stain itself must have enough 'useable' droplets on each side of its pattern to be suitable for determination methods. The droplets must be well formed (elliptical, symmetrical with well-formed leading edge), between 3mm and 8mm, fast upward travelling and in a location of '10 and 2' on a clock face relative to the bloodstain pattern. The second factor is stain measurement, which is influenced by the analyst's experience, attention to detail and equipment used. Both factors affect the angle of impact and consequently the AOO determination.

3.2.1 Shape and Form of Bloodstain

Research by de Bruin et al., (2011) described the factors affecting AOO determinations and ways in which to improve them. The article discussed three main components of AOO calculations: software used, bloodstain selection and external influences. In the software component, they determined that both *HemoSpat*© and *BackTrack*™ yielded relatively similar analysis thus concluding that both programs performed point of origin determinations equally well. It discussed the importance of selecting blood drops that had large surface areas and a more elliptical shape (smaller angle of impact) as they produced less deviation from the actual point of origin and gave more reliable results. Both these points are in agreement with previously accepted notions about stain selection. The last variable tested in the article was in relation to number of walls. It stated that using more than one wall for AOO determinations reduced the deviations in the X (distance from wall) and Z (height) direction. In summary the article acknowledged and further validate already known practises adopted by BPA analysts. Unfortunately, there were no new or novel techniques suggested or tested in this article and although it touched on a few important factors in stain selection, the content did not deliver any original "improvements to area of origin determinations" which was an overall disappointment.

For most BPA analysts the elliptical shape of bloodstains gives the most information in determinations of area of origin. However, an article by Kettner et al., (2015), chose to evaluate the value of information derived from the patterns of circular bloodstains which have been thought to hit adjacent surfaces at 90-degree angles and provide indications of the height of the object used to exert blunt force trauma. BPA analysts often use circular bloodstains as a quick and rough guide to estimate the height of an impact spatter however this article indicated this may not always be the case. The study demonstrated that two distinct patterns (clusters) of circular bloodstains were regularly found on an impact site after a single blow to a bleeding wound. The clusters were influenced by impact site-to-wall distance and did not directly reflect impact height level as a rapid tool for BPA reconstruction purposes, thus should be more used as a guide rather than a determining factor. The most notable feature of the study was that it discussed in great depth the formation of the two cluster stains which may in future help prevent erroneous bloodstain pattern analysis e.g. Interpreting the presence of two stains being the result of two impacts (Kettner et al., 2015).

An article by Adam (2012) investigated another facet of BPA: bloodstain formation. The work aimed to examine the stains formed following an impact on paper of blood droplets in the millimetre size range and travelling at a variety of angles and velocities. It also reviewed the current theoretical models for spreading and splashing of liquid drops on surfaces. During bloodstain formation at the point of impact, a lamella (boundary layer of liquid) is created when the droplet comes in contact with a surface. This layer spreads rapidly in a radial direction while the majority of the droplet remains spherical. This stage is followed by the rim of the lamella swelling as liquid flows outwards from the spherical reserve to create a largely flat, disc-like entity with a swollen rim. The material within the rim may then recede inwards until equilibrium is attained (Adam, 2012). The preceding stain formation has been ascribed into four phases: contact/collapse; displacement; dispersion and retraction, with fluid dynamic reviews having demonstrated a generic likeness in these stain formation phases regardless of fluid properties (Reynolds et al., 2009).

A review of the current theoretical models in the article by Adam (2012) concluded that there were fundamental mathematical expressions that enabled the understanding of bloodstain characteristics according to the principles of physics and mathematics thereby putting interpretation of bloodstain patterns on a sound theoretical basis. The study also showed that for perpendicular impact the interpretation of a single stain may provide information on its impact conditions, though, only within very limited ranges of velocities would the calculations of droplet size and impact speed be achievable to a good degree of accuracy. For non-perpendicular impact, the current models were concluded to give more of an "approximation" thus there remains a gap in rigorous theoretical examinations of such systems. It was proposed that predictions of droplet size and impact speed of non-perpendicular impact may be possible with appropriate modifications to the expression for the perpendicular model. The study highlighted limitations in calculations of impact angle from the stain aspect ratio and also suggested future research into the effect of surface properties on bloodstain formation.

3.2.2 Impact Angle of Bloodstain

The angle of droplet-to-surface impact, or alpha *(α)*, can be defined as the "acute or internal angle formed between the direction of a blood drop and the plane of the surface it strikes" (Reynolds et al., 2009). The calculation for the angle of impact relies upon the assumption that the blood droplet is spherical at the time of surface impact and that there must be a uniform transfer of the blood sphere's fluid content onto the surface to result in a geometric ellipse shape as demonstrated in Figure 9.

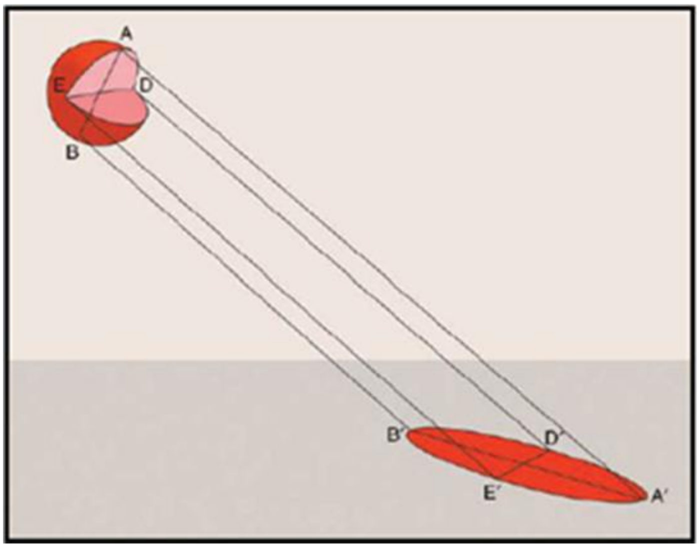

Figure 9 Diagrammatical representation of angle of impact calculation theory. Sphere is projected onto a surface as an elliptical stain (James et al., 2005)

Pace (2005) followed a similar path to the article by de Bruin et al., (2011) on reconstruction methods but only focused on one factor affecting area of origin determinations: impact angle. As mentioned above, BPA analysts abide by certain rules when selecting bloodstains for reconstruction purposes. Bloodstains between 3mm and 8mm are most often selected because they show the least deviation when using AOO calculation methods, a practice that is validated by a number of studies (Brodbeck, 2012, de Bruin et al., 2011, Peschel et al., 2011, Reynolds et al., 2009).

The article by Pace (2005) aimed to identify and investigate where sources of error may be introduced in AOO determinations and reflected on the error relationship between ellipse fitting and the increased degree of error in angle of impact calculations.

It was found that as the bloodstains became more rounded, i.e. angle of impact closer to 90°, the error rate became more pronounced too (Figure 10). Also, as the impact angle increased the more prominent 1mm errors in length measurements became (Table 2).

Table 2 Table showing the increase in impact angle calculation error by the same amount of measurement error over the range of impact angles 25°-70° (Pace, 2005)

Width	15	**15mm**	15	15	**15mm**	15	15	**15mm**	15	15	**15mm**	15
Length	34	**35mm**	36	24	**25mm**	26	19	**20mm**	21	15	**16mm**	17
Angle of impact	26.2°	**25.4°**	24.6°	38.7°	**36.9°**	35.2°	52.1°	**48.6°**	45.6°	90.°	**69.6°**	61.9°
Error	0.8°		0.8°	1.8°		1.7°	3.5°		3.0°	20.4°		7.7°

Figure 10 supports previously held studies by de Bruin et al., (2011) which suggest choosing more elliptical blood droplets decreases deviation in AOO calculations. Table 2 shows the error in the angle of impact calculation is practically negligible up to impact angle of around 50°, thereafter it increases rapidly, a 2mm difference in ellipse length (highlighted) resulted in a 12.7° error margin. It is immediately apparent therefore, as the angle of impact increases, even a small measurement error will make a large difference in subsequent calculations (Pace, 2005).

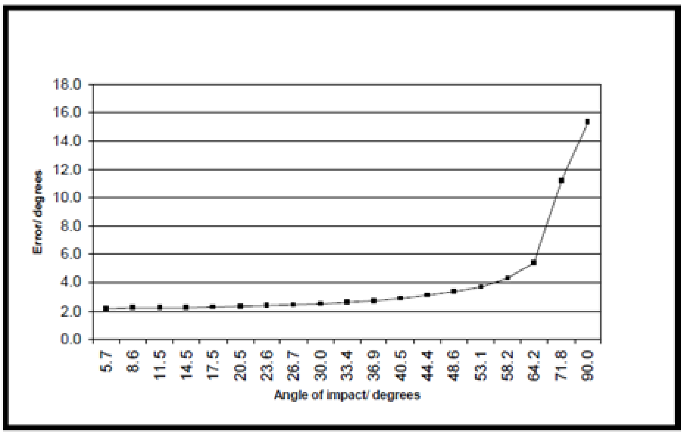

Figure 10 Graph showing how the error in calculating a bloodstain's angle of impact varies as a function of the bloodstain angle of impact itself (Pace, 2005)

3.2.3 Size of Bloodstain

In much of the literature relating to bloodstain pattern analysis and stain selection, bloodstain size is probably the least researched area of the field. Bloodstain size is one of the predominant factors influencing blood drop selection for area of origin determinations but has been neglected in terms of research. According to the formative literature by the likes of Balthazard et al., (1939), de Bruin et al., (2011) and Attinger et al., (2013) manual bloodstain measurement methods related to maximising accuracy and precision have seen a preferred selection of bloodstains for reconstruction, in part related to size. Bloodstains selected most often for these determinations are generally proportionally larger than other physically and geometrically suitable stains present within an impact pattern (Reynolds, 2008) due to the increased likelihood of manual measurement error as the droplets become smaller. Although there are a range of studies proving this premise to be correct there is no real evidence supporting the premise that larger bloodstains give more accurate results in comparison to smaller bloodstains. With the ever developing nature of BPA and technology such as digital photography and computer assisted ellipse fitting methods the range of bloodstains that can be included in reconstructions has significantly increased. This magnifies the research gap in bloodstain size selection and brings into question if larger bloodstains really are the most accurate selection method or if smaller bloodstains can be just as precise and accurate when used in conjunction with more novel technology.

An article by Reynolds et al., (2009) was the first of its kind to comparatively demonstrate the use of small versus larger bloodstains in area of origin determinations using a novel computer assisted program *Microsoft® Office Excel 2003 Auto Shape* and the industry standard program *BackTrack™ Images.* The article highlighted the literature gap regarding the role of inertial, viscous and surface tension forces during droplet impact and the formation of a bloodstain.

Studies have shown that internal pressure of any blood droplet is inversely proportional to radius and for very small droplets, the greatest influence on shape and consequently size, is surface tension (Raymond et al., 1996b). Reynolds et al., (2009) stated that due to angle of impact calculations being based on the length to width ratios of a bloodstain, any relative changes to that ratio due to the interplay of competing forces governing droplet spread, had the potential to influence bloodstain measurement outcomes. In order to compare the applied measurement resolution of small bloodstains against theoretical expectations, a two-part study was undertaken. The first part of the study analysed bloodstains caused by droplets falling vertically (under the influence of gravity alone) onto a range of inclined surfaces with known angles. The results showed that for small bloodstains (<3mm long) impacting a surface at angles <20°, the calculated angles of impact did not agree with theoretical expectations. In fact, it showed that deviation increased as the obliqueness of the impact angle increased (Figure 11). The small bloodstains consistently showed an over-estimation of impact angle which would be as a direct result of under-estimations of ellipse length relative to ellipse width. Subsequently this would mean that the calculated area of origin would be further away than the theoretical expected area of origin. Previous studies however have only ever reported over-estimations of ellipse length and consequently under-estimations of impact angles resulting in area of origins to be calculated as closer than expected area of origin (Connolly et al., 2012, de Bruin et al., 2011, Illes and Boue, 2011).

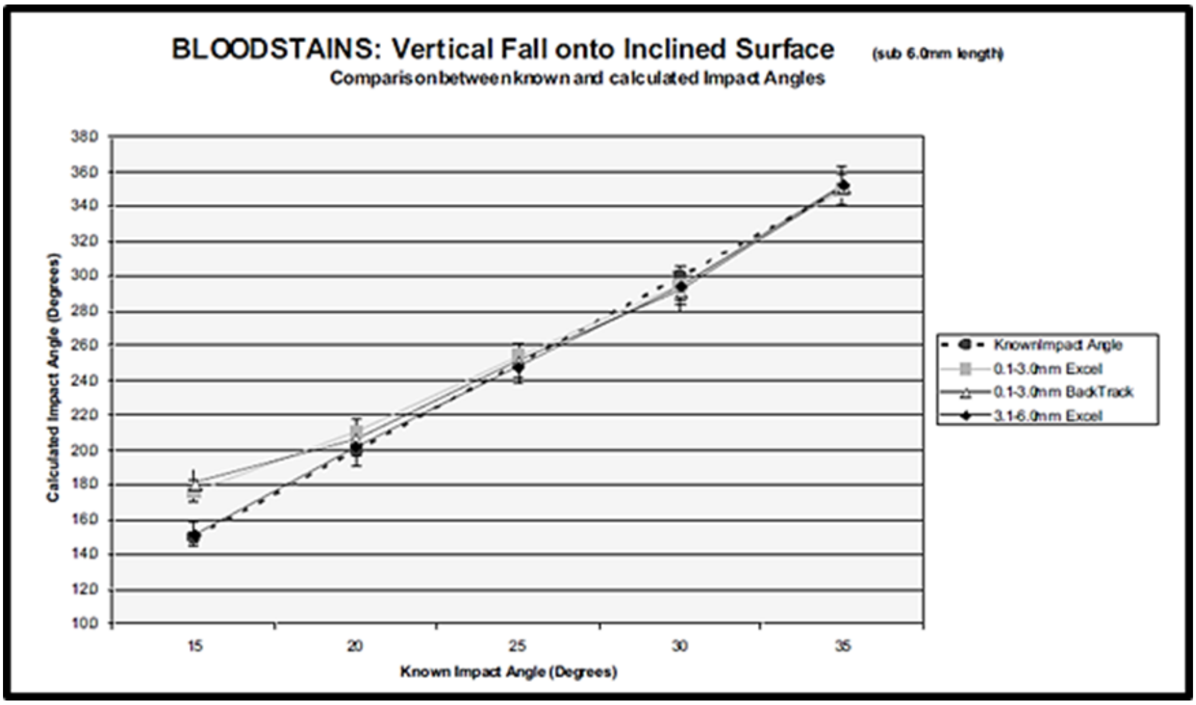

Figure 11 Comparison between known and calculated angles of impact (α) for bloodstains ≤3.0mm long and >3.0mm and ≤6.0mm long that have fallen onto ceramic tiles at known impact angles. (Reynolds et al., 2009)

For the second part of the study, when small bloodstains (sub 3mm) were used in AOO determinations the results demonstrated positive X co-ordinate values for seven out of the ten patterns (70%) *i.e.* an over-estimation of impact angles. Of these seven patterns, 65% had calculated impact angles of ≤25.0°. In comparison, of the three patterns with equal or negative X co-ordinate values only 28% of calculated impact angles were ≤25.0°. When larger bloodstains were used for comparison purposes, results showed negative or equal X co-ordinate values for all ten patterns. This is agreement with previous studies of larger bloodstains that demonstrate the same result regarding under-estimation of impact angles resulting in AOO determinations to be closer than the theoretical AOO. Of the larger bloodstains used in AOO calculations, 60% of calculated impact angles were ≤25° but as expected the length and width of the ellipses were proportionately larger to the small bloodstains used. When compared to each other the percentage of bloodstains with calculated impact angles ≤25° between the smaller size bloodstain sample set and the larger size set was essentially the same; 65% and 60% respectively.

Although the study was unable to pinpoint the exact cause of this apparent shift in X co-ordinate values, it did suggest likely causes; "results suggest the variance is associated to changes in relative influences of competing forces namely inertia, viscosity and in particular surface tension during droplet to bloodstain transition" (Reynolds et al., 2009). Furthermore, it stated that as blood droplet size and surface impact angle decreased, the relative influence of surface tension on stain formation increased, which resulted in the over-estimations of calculated impact angles.

The study concluded that until further research was conducted into identification and quantification of the influences of the aforementioned forces on the formation and geometric display of small blood drops they should not be used for AOO reconstruction purposes. The study was very thorough in its experimental design and conclusions however; it could have included more on the limitations to the study. For example, the use of tiles modified to mimic different angles of impact and subsequently dried horizontally did not reflect real life conditions. This method of using passive droplets only took into consideration the effect of gravity on droplet formation and neglected drag and inertial forces. The method of horizontally drying the tiles after droplet impact would have influenced the spreading and drying of the stain to occur differently from if it was drying vertically on a wall. Given however, that this was one of the first research papers to suitably investigate a core bloodstain selection process it was thorough and precise in its findings. The need for further validating research is however apparent and mentioned within the article.

As can be seen from the aforementioned articles, there was a vast expanse of research conducted in the BPA field. The research however, did not thoroughly reflect or sufficiently encompass bloodstain selection or its criteria. As one of the most fundamental parts of BPA and AOO determinations it seemed negligent that justifications and further research into the stain selection criterion had not been adequately provided. A research gap was evident and should be rectified with future research.

4. Proposed Research

The aim of my research is to further investigate the effectiveness of small (sub 3mm) bloodstains in AOO determinations and visualise the apparent shift in influences of inertia and surface tension on increasingly oblique impact angles. The null hypothesis is that there will be no significant difference in the small (<3mm) bloodstains when compared to the larger (>3mm) bloodstains. The specific objectives will be to comparatively determine if;

1. Sub 3mm bloodstains can be used as effectively as the industry accepted standard (between 3mm and 8mm) bloodstains in AOO determination.

2. Deviation from actual AOO increases in sub 3mm droplets as the impact angles become increasingly oblique.

To do this, all stain widths, lengths and impact angles will be generated using the *Microsoft® Office Excel 2003 Auto Shape* program. Subsequently, the AOO will be determined using the Tangent Method.

The AOO determinations for each of the ten patterns as replicates will be statistically analysed using the Analysis of Variance (ANOVA) statistical test in MS Excel where comparisons will be made between the small (sub 3mm) bloodstains and >3mm bloodstains as well as with the true AOO for each pattern. Depending on the results of the ANOVA, post-hoc tests may or may not be undertaken.

5. Glossary

Listed below are terms used in this literature review. Definitions have been adapted from the SWGSTAIN Terminology Manual (2013) unless otherwise stated.

Angle of Impact
The acute angle (alpha), relative to the plane of a target, at which a blood drop strikes the target

Area of Origin
The three- dimensional location from which spatter originated

Bloodstain
A deposit of blood on a surface

Bloodstain Pattern
A grouping or distribution of bloodstains that indicates through regular or repetitive form, order or arrangement the manner in which the pattern was deposited

Flight path
The path of the blood drop as it moves through space from the impact site to the target (Reynolds, 2008)

Impact Pattern
A bloodstain pattern resulting from an object striking liquid blood

Passive drop
A bloodstain drop created or formed by the force of gravity acting alone

Spatter Stain
A bloodstain pattern resulting from a blood drop dispersed through the air due to an external force applied to a source of liquid blood

Target
A surface onto which blood has been deposited

Terminal Velocity
The maximum speed to which a free-falling drop of blood can accelerate in air which is approximately 25ft/sec (Reynolds, 2008)

6. References

ADAM, C. D. 2012. Fundamental studies of bloodstain formation and characteristics. *Forensic Sci Int,* 219**,** 76-87.

ATTINGER, D., MOORE, C., DONALDSON, A., JAFARI, A. & STONE, H. A. 2013. Fluid dynamics topics in bloodstain pattern analysis: comparative review and research opportunities. *Forensic Sci Int,* 231**,** 375-96.

BALTHAZARD, V., PIEDELIEVRE, R., DESOILLE, H. & DEROBERT, L. 1939. Etude des Gouttes de Sang Projecte (Study of Projected Drops of Blood). *Ann Med Leg Criminol Police Sci Toxicol,* 19**,** 265-323.

BRODBECK, S. 2012. Introduction to Bloodstain Pattern Analysis *Journal for Police Science and Practice* 2**,** 51-57.

BUCK, U., KNEUBUEHL, B., NATHER, S., ALBERTINI, N., SCHMIDT, L. & THALI, M. 2011. 3D bloodstain pattern analysis: ballistic reconstruction of the trajectories of blood drops and determination of the centres of origin of the bloodstains. *Forensic Sci Int,* 206**,** 22-8.

CARTER, A. L. 2001. The Directional Analysis of Bloodstain Patterns- Theory and Experimental Validation *Canadian Society of Forensic Science* 34**,** 173-189.

CARTER, A. L., FORSYTHE-ERMAN, J., HAWKES, V., ILLES, M., LATURNUS, P. L., LEFEBVRE, G., STEWART, C. & YAMASHITA, A. B. 2006. Validation of the Backtrack™ suite of programs for bloodstain pattern analysis. *J Forensic Identification,* 56**,** 242-245.

CARTER, A. L., ILLES, M., MALONEY, K., YAMASHITA, A. B., ALLEN, B., BROWN, B., DAVIDSON, L., ELLIS, G., GALLANT, J., GRADKOWSKI, A., HIGNELL, J., JORY, S., LATURNUS, P. L., MOORE, C. C., PEMBROKE, R., RICHARD, A., SPENARD, R. & STEWART, C. 2005. Further validation of the Backtrack™ computer program for bloodstain pattern analysis-precision and accuracy. *IABPA News,* 3.

CONNOLLY, C., ILLES, M. & FRASER, J. 2012. Effect of impact angle variations on area of origin determination in bloodstain pattern analysis. *Forensic Sci Int,* 223**,** 233-40.

DE BRUIN, K. G., STOEL, R. D. & LIMBORGH, J. C. 2011. Improving the point of origin determination in bloodstain pattern analysis. *J Forensic Sci,* 56**,** 1476-82.

ILLES, M. & BOUE, M. 2011. Investigation of a model for stain selection in bloodstain pattern analysis. *Canadian Society of Forensic Science,* 44**,** 1-12.

JAMES, S., KISH, P. & SUTTON, T. P. 2005. *Principles of bloodstain pattern analysis: theory and practice* Boca Raton, FL, CRC Press.

KABALIUK, N., JERMY, M. C., WILLIAMS, E., LABER, T. L. & TAYLOR, M. C. 2014. Experimental validation of a numerical model for predicting the trajectory of blood drops in typical crime scene conditions, including droplet deformation and breakup, with a study of the effect of indoor air currents and wind on typical spatter drop trajectories. *Forensic Sci Int,* 245**,** 107-20.

KETTNER, M., SCHMIDT, A., WINDGASSEN, M., SCHMIDT, P., WAGNER, C. & RAMSTHALER, F. 2015. Impact height and wall distance in bloodstain pattern analysis--what patterns of round bloodstains can tell us. *Int J Legal Med,* 129**,** 133-40.

MACDONELL, H. 1971. Flight Characteristics and Stain Patterns of Human Blood. *US Dept of Justice NIJ Pub***,** 77.

PACE, A. 2005. The relationship between errors in ellipse fitting and the increasing degree of error in angle of impact calculations. *IABPA News,* 3**,** 12-14.

PESCHEL, O., KUNZ, S. N., ROTHSCHILD, M. A. & MUTZEL, E. 2011. Blood stain pattern analysis. *Forensic Sci Med Pathol,* 7**,** 257-70.

PIOTROWSKI, E. 1895. Ueber Entstehung, Form, Richtung und Ausbreitung der Blutspuren nach Hiebwunden des Kopfes [On the formation, form, direction, and spreading of blood stains after blunt trauma to the head].

RAYMOND, M. A., SMITH, E. R. & LIESEGANG, J. 1996. The physical properties of blood--forensic considerations. *Sci Justice,* 36**,** 153-60.

REYNOLDS, M. 2008. *Bloodstain Size, Shape and Formation; implications for the bloodstain pattern analyst.* Masters of Forensic Science/ Doctor of Philosophy, University of Western Australia.

REYNOLDS, M., RAYMOND, M. A. & DADOUR, I. 2009. The use of small Bloodstains in Blood Source Area of Origin Determinations. *Canadian Society of Forensic Science,* 42**,** 133-146.

Page left intentionally blank

Part Two

Project Manuscript

The Effectiveness of Small (sub 3mm) Bloodstains on Area of Origin Determinations

Abstract

The use of bloodstain pattern analysis (BPA) in reconstruction of bloodletting events has become a critical and integral part of many criminal investigations and court cases. In instances where a bloodstain pattern is created due to external forces being applied to a source of blood, an impact spatter pattern is created. Traditionally, BPA analysts have followed a rigid protocol for selection of bloodstains used in these reconstruction events in attempts to lessen sources of error that directly impact area of convergence (AOC) and area of origin (AOO) calculations. One such selection criteria is bloodstain size; bloodstains that have a length (l) between 3mm and 8mm are considered useful for reconstruction determinations. The purpose of this study was to evaluate the effectiveness of small (l <3mm) bloodstains in AOO determinations in comparison to the accepted industry standard and identify the involvement of impact angles in these calculations. Bloodstain impact angles and AOO calculations were conducted using the *Microsoft® Office Excel 2003 Auto Shape* program and tangent method respectively. Bloodstain size was found to have no impact on X (p-value=0.906), Y (p-value=0.262) and Z (p-value=0.688) co-ordinates for AOO determinations and decreasing angles of impact showed no correlation to deviation from the actual AOO.

Keywords: droplet size, area of origin, bloodstain pattern analysis, impact angle, ellipse, forensic science

1. Introduction

In Western Australia the number of violent crimes accounted for 77% of total offences against a person from June 2015 to June 2016 (WAPOL Crime Statistics Portal). Furthermore, according to the Australian Bureau of Statistics, in 2015 homicide rates in WA increased by approximately 33% since the previous year and assault increased by 17% since 2014, representing the largest recorded increase over a 12-month period in any state or territory in Australia. Naturally with the increasing number of violent crimes, there has also been an increasing demand for forensic specialties such as bloodstain pattern analysis (BPA). BPA has been used for centuries in the determination of circumstances resulting in a bloodletting event (Attinger et al., 2013). It has proved to be a critical and sometimes pivotal piece of evidence that help the finders of fact piece together events of a violent and at times, fatal incident.

Among the array of bloodstain patterns that can be distinguished, an impact spatter is of particular evidentiary value and forensic interest (Peschel et al., 2011). Impact spatter is described by the Scientific Working Group on Bloodstain Pattern Analysis (SWGSTAIN) as, *"a bloodstain pattern resulting from an object striking liquid blood"*. The radiating pattern of individual droplets that results from this can help investigators in spatial, temporal and contextual reconstruction of the events resulting in bloodshed.

Area of Origin (AOO) determinations are of particular forensic interest as they define the three dimensional location where a source of blood originated. The width and length of selected stains within an impact pattern result in the determination of impact angles *(α).*

The droplets can then assist in area of convergence (AOC) determinations by lines drawn from the major axis of selected droplets to an area where they intersect each other in a 2D form. AOO is an extension of this method by which the AOC location along with impact angles from selected bloodstains result in a 3D source location from where the blood originated.

This 3D source location is denoted the co-ordinates X, Y and Z (Figure 12) where;

- X represents the blood source distance from target surface (horizontal distance to a front wall
- Y represents the blood source distance from a horizontal reference point (distance to a side wall)
- Z represents the blood source height (vertical distance from AOC to floor)

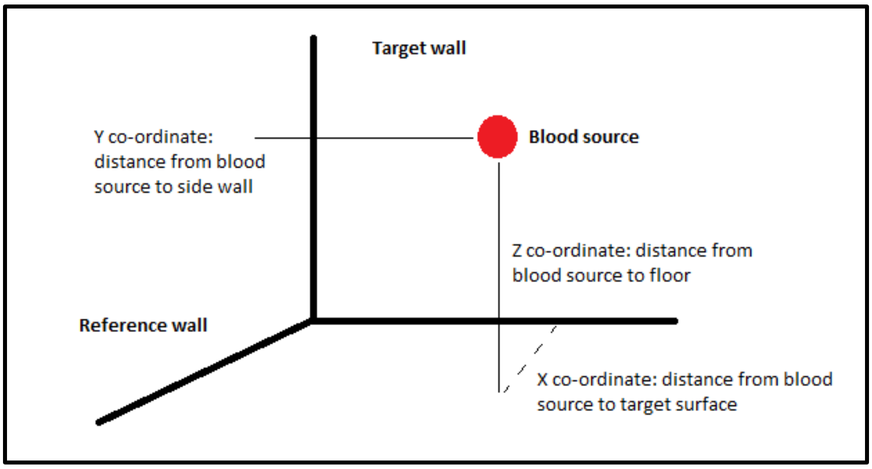

Figure 12 3D diagrammatical representation of AOO in relation to X, Y and Z co-ordinates (image by Brinda Salaskar)

AOO determinations are based on Euclidean geometry and straight line blood droplet flight path predictions. In other words, the methods are predicated on the assumption that the path of a blood drop away from the blood source follows a straight line trajectory, thus eliminating the effects of gravity and drag. The accuracy of AOO determination relies upon the analyst implementing a strategic sampling rationale based on the spatial and geometric characteristics of the spattered bloodstains present within the impact pattern (Reynolds, 2008). Consequently, there are specific characteristics of blood droplets sought out by BPA analysts in their AOO determinations that help minimise or reduce these effects of error and increase accuracy. One such selection criteria is blood droplet size; blood droplets with lengths (l) of between 3mm and 8mm are considered useful in determination methods. Other selection criteria include multiple bloodstain selection from both sides of an impact pattern, shape (well-formed elliptical stains) and location in relation to the spatter bearing surface (fast upward travelling droplets that lie between the '10 and 2' location on a clock face)(Carter et al., 2005, James et al., 2005, Carter et al., 2006, Bevel and Gardner, 2008, Wonder and Yezzo, 2015). Whilst experimentally these criteria can be controlled, in the real world crime scenes don't always follow these "perfect conditions" and other scenarios are constantly presented and tested. Smaller blood droplets (l <3mm) are being considered with the ever growing power of digital photography and advancements in measurement and reconstruction software (Reynolds et al., 2009, Reynolds, 2008).

There are substantial amounts of information regarding straight-line trajectories of blood droplets in flight (Macdonell, 1971), droplet deformation (Kabaliuk et al., 2014) and impact angle influences on reconstruction (Adam, 2012) but minimal research regarding the selection criteria for AOO reconstruction *i.e.* blood drop size and its effects on AOO determinations. A paper by de Bruin et al., (2011) briefly mentioned the influence of droplet size in their research into improving point of origin determinations and a thesis by

Evans (2015) investigated the effects of haematocrit values of blood on AOO determinations. But it appears that within all the available research regarding AOO determinations there is a research gap in the factors influencing droplet selection, namely droplet size. The most recent if not only paper that discussed the influence of droplet size in AOO determinations arose in 2009 by Reynolds et al., where a focal research point was using small bloodstains for AOO determinations using the existing industry standard computer assisted method *BackTrack™ Images* and a novel program *Microsoft® Office Excel 2003 Auto Shape*. The research concluded that the use of digital photography and computer assisted methods had broadened the range of bloodstain sizes that were able to be used in determinations however, reflected that previously accepted notions that bloodstains with a larger surface area corresponded to less deviation still held true. The paper also mentioned that extrinsic and intrinsic forces affected the AOO calculations for small (l<3mm) bloodstains that impacted surfaces obliquely at low impact angles (α<15°) resulting in a positive direction of difference between the calculated X value and actual blood source X value. This was opposed to the commonly calculated negative blood source X value determinations. The hypothesised reason for this was that surface tension, inertia and viscosity were a greater influence on the smaller droplets as they transitioned from droplets in flight to bloodstains on an impact surface.

The ensuing study follows the research conducted by Reynolds et al., (2009) and aimed to investigate the effectiveness of small (l<3mm) blood droplets in AOO determinations, where the null hypothesis showed no statistically significant differences between determination of AOO for bloodstains <3mm in length and the accepted method (3mm to

8mm) for X, Y and Z values. Furthermore, it aimed to evaluate an observation put forward in the article by Reynolds et al., (2009) where there was an overestimation of X co-ordinate values in small bloodstains with impact angles of <15°. The null hypothesis showed no statistically significant difference between X co-ordinate values for small bloodstains with angles of impact <15° and actual X co-ordinate values.

For comparative purposes large blood droplets (l>3mm) were also selected and impact spatter patterns were created by a method of blind trials. The X, Y and Z co-ordinates were unknown to the author until all statistical and computational analyses had been completed. Deviation from actual AOO in sub 3mm droplets as impact angles became increasingly oblique was also briefly explored.

2. Materials and Methods

Pattern Creation

Bloodstain patterns were created using fresh (<5hrs old) whole human blood stored in EDTA anti-coagulant tubes. Ten impact patterns were created in BPA demountable rooms at Murdoch University by striking the head of a claw hammer onto a pool of blood (~5mL) placed on wooden block apparatuses of varying heights (40cm and 65cm) (Figure 13). The impact spatters were deposited onto 104cm x 156cm semi-gloss (880GSM) screen boards affixed to the wall using all-purpose masking tape.

A BPA analyst from the Western Australian Police Forensic team generated the patterns using consistent methodology for all ten patterns and recorded the X, Y and Z co-ordinates for each source location. Each source location was varied in dimensions and produced by a process of ‘blind trials’ so as to ensure no investigator bias was encountered. This approach ensured all analyses conducted in this experiment relied solely on the stain selection and calculated dimensions from the impact patterns created.

Figure 13 Wooden apparatus and hammer used to create impact spatters (Evans, 2015)

Stain Selection and Photography

Once the ten impact patterns were created and allowed sufficient time for drying (~15mins), the BPA expert made a selection of ten droplets from each pattern that were consistent with the selection criterion used by BPA analysts in WA Police. Selection included well-formed fast upward travelling droplets that were appropriately distributed, symmetrical, elliptical and between 3mm-8mm in length. Two study analysts (including the author) then selected ten droplets with identical selection criteria bar the length of the droplets which were limited to below 3mm. A scale and reference number was then added next to each stain and photographed using a Nikkon D5500 digital camera with an AF-S Micro Nikkor 60mm lens. The micro lens aperture was calibrated at f/4.0 with an ISO of 800 and shutter speed of 1/250 seconds. Figure 14 shows the types of technical photographs taken of each stain in this study.

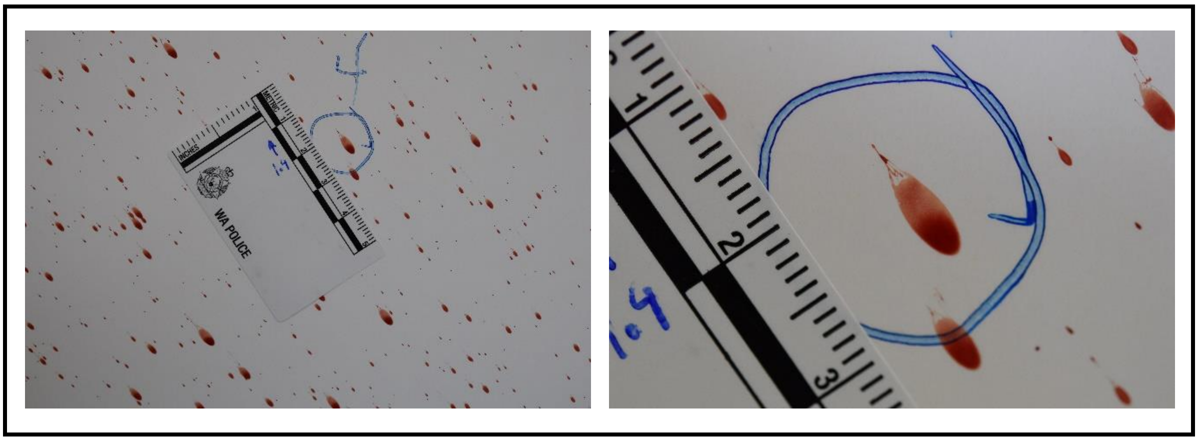

Figure 14 Close up and technical photography of stain with scale using the Nikkon D5500 digital camera

Data Analyses

Each stain was subsequently analysed using the *Microsoft® Office Excel 2003 Auto Shape* program to determine its impact angle *(α)* using the equation:

$$Impact\ angle\ (\alpha) = sin^{-1}\left(\frac{width}{length}\right) \qquad [1]$$

The *Microsoft® Office Excel 2003 Auto Shape* program was chosen because it had the capability to calculate impact angles to sub-degree levels and the ability to symmetrically auto-elongate the measuring ellipse during the fitting process, ensuring both halves of the ellipse were mirrored. This retained the integrity of the ellipsoid's mathematical properties (Reynolds, 2008) and made the program unique among its predecessors Backtrack™ and Hemospat® for its ability to calculate ellipses to sub degree levels (de Bruin et al., 2011, Reynolds, 2008). Macro functions such as the scaling bars were another important function of the program as they accounted for distortion error associated with photography of the bloodstain. Figure 15 shows the fitting process of a bloodstain used in this study using the *Microsoft® Office Excel 2003 Auto Shape* program.

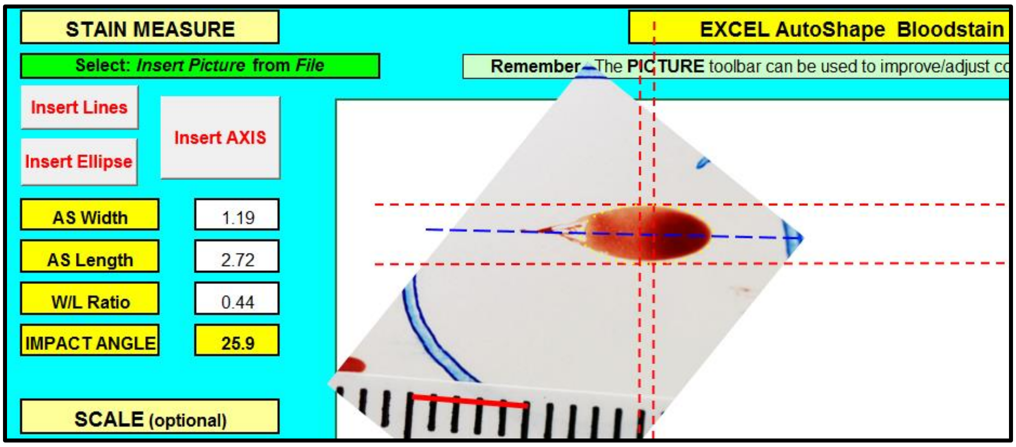

Figure 15 Bloodstain used in the *Microsoft® Office Excel 2003 Auto Shape* program with axis, fitted ellipse and scale added (image by Brinda Salaskar)

After photography, the blood droplets were grouped into small and large stains and using different colours, a directional line was drawn through the long axis of each selected blood droplet to an area where it reached its maximum length (in this case, to the bottom of the screen board). Where the lines of each of the stains intersected on another was determined to be the point of convergence for that group (small or large) on the pattern. The point of convergence to the floor was determined to be the height for each pattern (Z co-ordinate) and the point of convergence to the side wall was determined to be the Y co-ordinate.

The Tangent method calculated the distance from the pattern bearing surface (X co-ordinate) for each pattern first using the ten small blood droplets (l <3mm) and subsequently the ten larger blood droplets (l>3mm) with the equation:

$$Tan\ (\alpha) = \frac{opposite}{adjacent} \qquad [2]$$

These values were then averaged to determine the final X co-ordinate values for the small and large bloodstain groups.

The known X, Y and Z co-ordinates were subtracted from the experimental results in order to derive the directionality of difference *ie.* positive or negative, and subsequently the analysis of variance (ANOVA) statistical test on Microsoft Excel was used to calculate the variance among the AOO for each pattern, namely the experimental small (S), experimental large (L) and the known X, Y and Z co-ordinates. The data was critically assessed at a significance level of 0.05 and the mean, standard deviation and absolute values for the variables (X, Y and Z co-ordinates) were also recorded. Post-hoc paired t-tests were used as a secondary measure of comparison for each variable to determine if there were any significantly reportable variances among the small, large and known results.

3. Results

Statistical analysis of ten sets of generated BPA bloodletting events was undertaken between and within each impact spatter pattern group. The data is presented in terms of average differences between experimental and known results due to the differing X, Y and Z co-ordinates for each pattern. For each co-ordinate, an average mean, standard deviation and absolute value were calculated for comparative purposes (Table 3) and the calculated values vs actual values are presented in Figures 16-18. Small bloodstain size ranged from 0.61mm-1.81mm in width and 1.42mm-2.98mm in length and large bloodstain size ranged from 1.12mm-6.53mm in width and 3.12mm-8.36mm in length across the ten patterns. Impact angles ranged from 17.3°-66.1°. The experimental X, Y and Z means represented the displacement from the known co-ordinates (X, Y and Z) and were presented negative or positive values (Figures 19 and 20). Negative values signify an underestimation of the experimental co-ordinate compared to the known value and positive values represent an overestimation (Reynolds et al., 2009). Standard deviation represents the spread or dispersion of the groups and absolute values represent the average displacement of the group as a whole irrespective of direction of difference (positive or negative).

Table 3 Shows *Microsoft® Office Excel 2003 Auto Shape* measurement data for large bloodstains ℓ >3mm and small bloodstains ℓ< 3mm **(IN BRACKETS AND BOLDED)** for area of origin determinations for impact patterns 1-10.

Pattern	Stains (n)	Co-ordinates	Known Value (cm)	Experimental Value (cm)	Difference (cm)
1	10	X	34	37.8 **(32.7)**	3.8 (-1.3)
		Y	146	144.9 **(145.0)**	-1.1 (1.0)
		Z	65	66.6 **(67.5)**	1.6 (2.5)
2	10	X	69	61.9 **(63.8)**	-7.1 (-5.2)
		Y	139	140.6 **(140.6)**	1.6 (1.6)
		Z	65	77.2 **(80.0)**	12.2 (15.0)
3	10	X	46	42.6 **(44.8)**	-3.4 (-1.2)
		Y	130	131.6 **(132)**	1.6 (2.0)
		Z	40	43.5 **(44.5)**	3.5 (4.5)
4	10	X	49	47.2 **(48.4)**	-1.8 (-0.6)
		Y	181	180.2 **(179.5)**	-0.8 (-1.5)
		Z	65	68.4 **(68.5)**	3.4 (3.5)
5	10	X	78	80.6 **(75.6)**	2.6 (-2.2)
		Y	152	154.5 **(153.3)**	2.5 (1.3)
		Z	65	66.8 **(67.5)**	1.8 (2.5)
6	10	X	72	75.0 **(76.5)**	3.0 (4.5)
		Y	112	124.3 **(125.0)**	12.3 (13.0)
		Z	40	55.3 **(52.4)**	15.3 (12.4)
7	10	X	38	37.7 **(42.0)**	-0.3 (4.0)
		Y	121	117.5 **(117.5)**	-3.5 (-3.5)
		Z	40	40.0 **(36.1)**	0.0 (-3.9)
8	10	X	52	51.8 **(52.9)**	-0.2 (0.9)
		Y	119	119.4 **(119.1)**	0.4 (0.1)
		Z	65	67.0 **(66.2)**	2.0 (1.2)
9	10	X	63	61.2 **(61.5)**	-1.8 (-1.5)
		Y	130	129.9 **(134.2)**	-0.1 (4.2)
		Z	40	53.7 **(55.0)**	13.7 (15.0)
10	10	X	40	42.0 **(40.6)**	2.0 (0.6)
		Y	108	107.1 **(110.5)**	-0.9 (2.5)
		Z	40	47.0 **(50.9)**	7.0 (10.9)
Mean		X	-0.32 **(-0.21)**		
		Y	1.20 **(1.87)**		
		Z	6.05 **(6.36)**		
Standard Deviation		X	3.37 **(3.84)**		
		Y	4.26 **(4.49)**		
		Z	5.65 **(6.50)**		
Absolute Value		X	2.60 **(2.81)**		
		Y	2.66 **(3.13)**		
		Z	6.05 **(7.14)**		

Table 4 ANOVA statistical data for variances between small, large and actual X, Y and Z values for impact patterns 1-10

	F-value	Degrees of freedom	P-value
X co-ordinate	0.015	1	0.906
Y co-ordinate	1.431	1	0.262
Z co-ordinate	0.172	1	0.688

Table 5 Paired t-test data for variances between experimental (large and small) and actual X, Y and Z values for impact patterns 1-10

X co-ordinate			
	Large v Actual	Small v Actual	Large v Small
P-value	0.769	0.825	0.906
Deg. of freedom	9	9	9
T-stat	-0.303	-0.227	0.121
Y co-ordinate			
P-value	0.396	0.221	0.262
Deg. of freedom	9	9	9
T-stat	-0.891	-1.316	-1.196
Z co-ordinate			
P-value	0.008	0.013	0.688
Deg. of freedom	9	9	9
T-stat	-3.387	-3.096	-0.414

Upon completion of the tangent method analyses, the following absolute displacement from actual values for large stains were obtained: X (large)= 2.60cm ±0.63cm, Y (large)= 2.66cm ±1.26, Z (large)=6.05cm ±1.79cm. From these large bloodstains, further analyses showed that the experimental X co-ordinate displacement from the known X value was approximately 5% and was underestimated on average by about 0.32cm across all ten patterns. The experimental Y co-ordinate showed a displacement from the known Y value by about 2% and a 1.2cm overestimation across all ten patterns. The Z co-ordinate showed the most difference in experimental versus known results, its displacement was approximately 13% from the known value and was overestimated on average by about 6.05cm.

The same method of analyses was applied to the small bloodstains from the ten impact patterns and the following absolute X, Y and Z displacement values were obtained: X (small)= 2.19cm ±0.55cm, Y (small)= 3.13cm ±1.30cm, Z (small)= 7.14cm ±1.75cm. Further analyses showed there to be an X co-ordinate displacement of approximately 4% and a 0.21cm underestimation on average among the ten patterns. The Y co-ordinate showed a 3% displacement of experimental results when compared to the known values and an overestimation of 1.87cm on average. The experimental Z value showed a proportionately larger displacement of 15% from the known results and was overestimated by approximately 6.36cm across the ten patterns.

ANOVA was subsequently carried out on the X, Y and Z co-ordinates with a statistical significance value of 0.05. The ANOVA statistical test results presented in Table 4 demonstrate the analysis of variance among the given co-ordinate's calculated large, calculated small and actual bloodstain values. The X co-ordinate showed no significant differences among the tested variables with a p-value=0.906, as did the Y co-ordinate with a p-value=0.262 and the Z-co-ordinate with a p-value=0.688.

Further analysis was conducted using two tailed paired t-tests for each of the co-ordinates. A paired t-test was used due to the systemic relationships that exist between the small, large and actual bloodstain values and was two tailed because there was a possibility a statistically significant relationship existed in either direction. For the Z co-ordinate a one tailed t-test was also carried out due to the assumptions of height always being overestimated in AOO determinations (Buck et al., 2011). The paired t-tests results for each co-ordinate were calculated using a significance level of 0.05 and compared actual values against each experimental value (small and large) and experimental values against each other. The paired t-test results are presented in Table 5. Only two of the nine paired t-tests rejected the null hypothesis to conclude a statistically significant difference in means; the Z co-ordinate *'Large v Actual'* and Z co-ordinate *'Small v Actual'* with p-values of 0.008 and 0.013 for the two tailed t-test and 0.004 and 0.006 for the one tailed paired t-test respectively.

There were no small bloodstains which generated impact angles of <15° in this study thus small bloodstains with impact angles of <25° were analysed. Four bloodstains were found to meet this criterion and originated from patterns one and ten. Pattern one had stain five, six and nine with impact angles of 21.6°, 24.8°and 23.7° respectively. Pattern ten had stain ten with an impact angle of 24.6°. Of these impact angles, two underestimated the X co-ordinate value (pattern one: stain five and six) and two overestimated it (pattern one: stain nine and pattern ten: stain ten). It was decided the dataset for small bloodstains with impact angles <25° was not large enough for any further statistical analysis or evaluation.

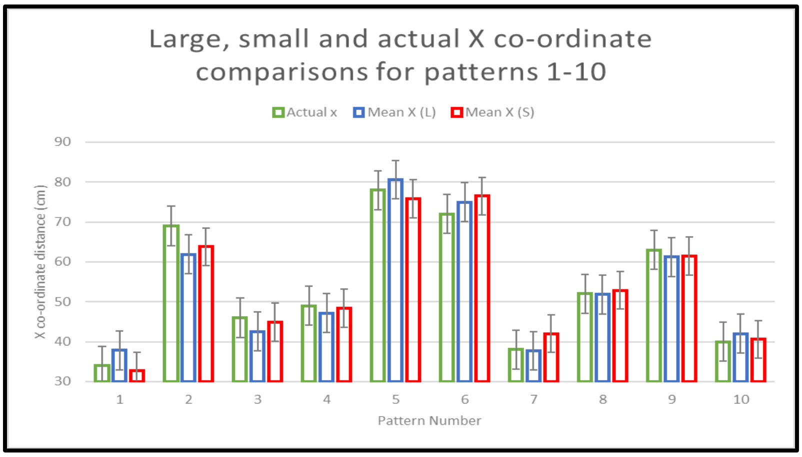

Figure 16 Large, small and actual X co-ordinate comparisons for patterns 1-10

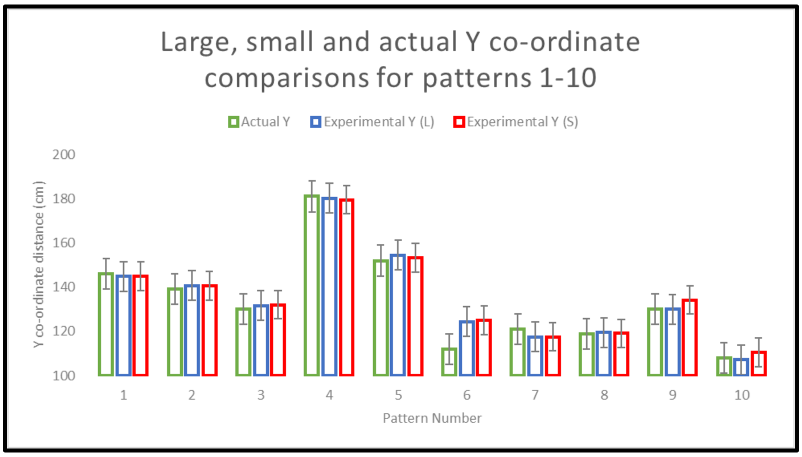

Figure 17 Large, small and actual Y co-ordinate comparisons for patterns 1-10

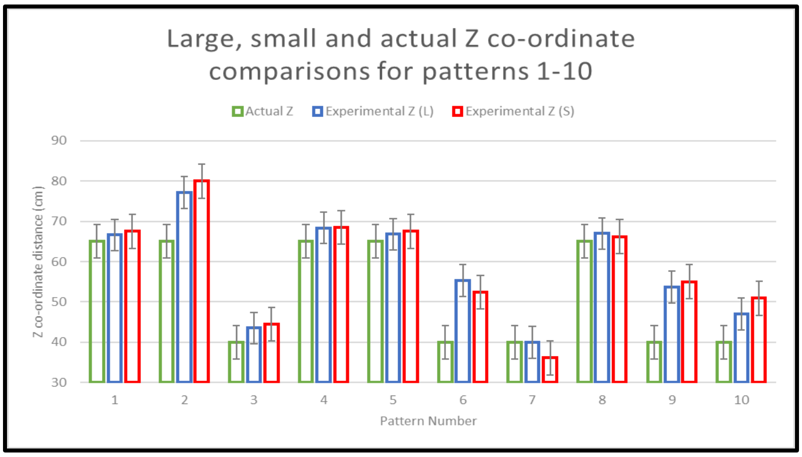

Figure 18 Large, small and actual Z co-ordinate comparisons for patterns 1-10

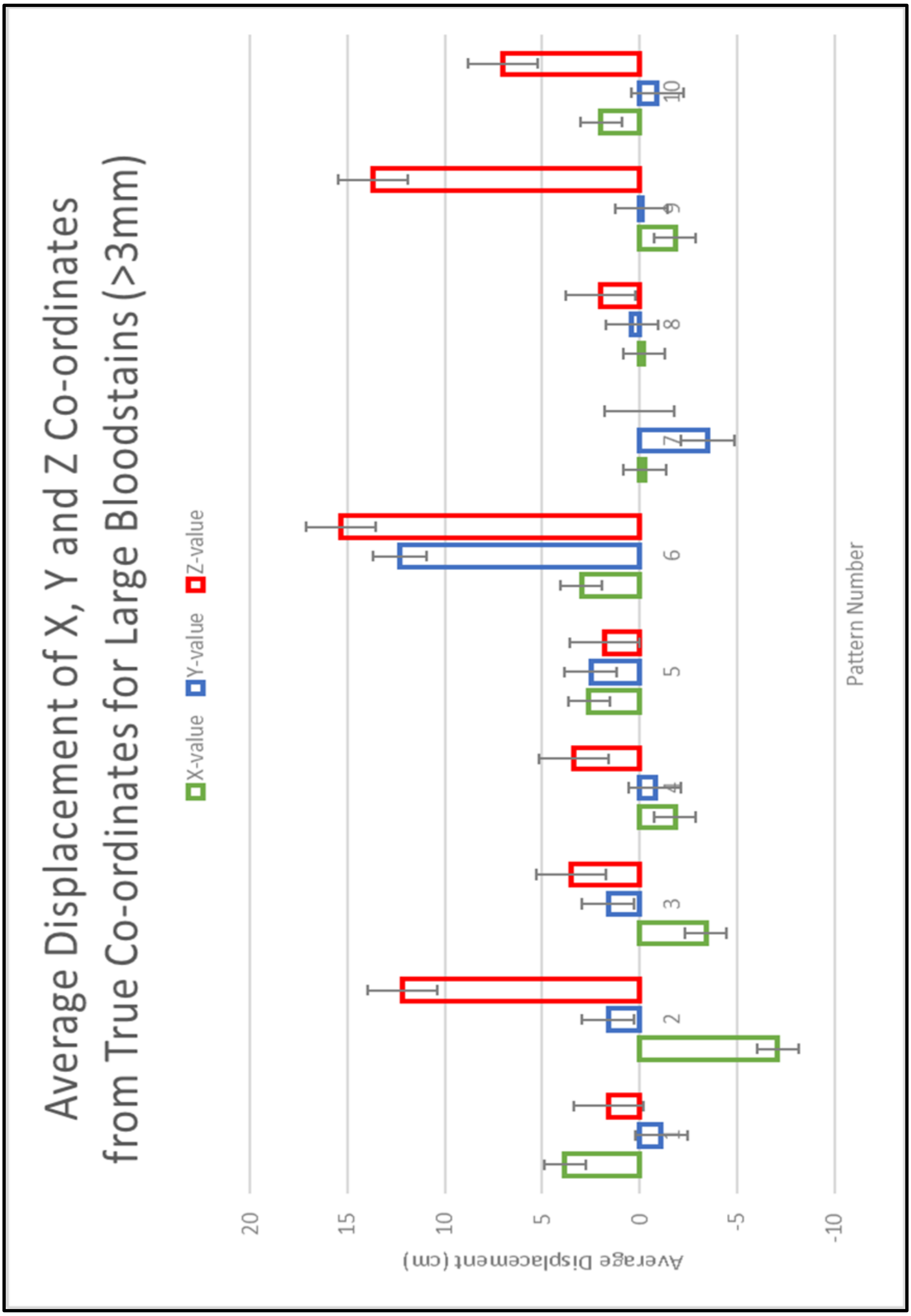

Figure 19 Average displacement of large bloodstains' calculated X, Y and Z co-ordinates from actual values for patterns 1-10

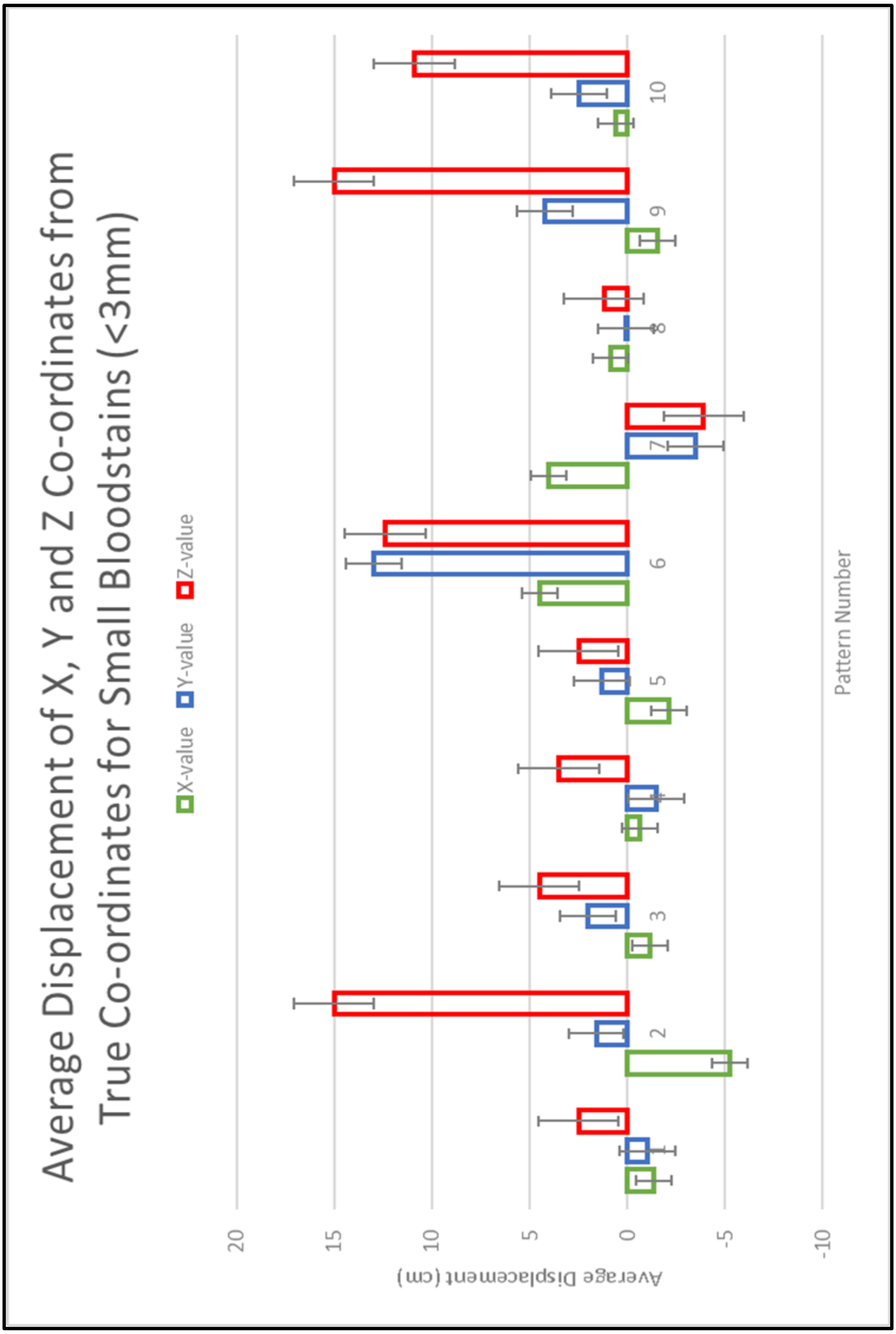

Figure 20 Average displacement of small bloodstains' X, Y and Z co-ordinates from actual values for patterns 1-10

4. Discussion

A research gap in the selection criteria used by BPA personnel in the reconstruction of bloodletting events has been identified in the course of reviewing the available BPA literature (Reynolds et al., 2009). Bloodstain size, shape and location are all critical pieces of the AOO puzzle; one without the other would result in the portrayal of only a snippet of the final picture. Historically, the acceptable size range for a bloodstain used in AOO determinations has been limited to be between 3mm-8mm in length (Illes and Boue, 2011, de Bruin et al., 2011). This originated from the article by Raymond et al., (1996) where size of blood droplets and their associated oscillation decay rates were investigated. The research found that droplets that impacted a vertical surface before oscillation had completed resulted in stains that predicted incorrect positioning of the droplet projection point (AOO). The study suggested using droplets of a distance >1m from AOO which was associated with larger droplets in the experiment. The study did not however investigate whether small droplets should not be used at these distances. Recently, the advancements made in digital photography and measurement software have allowed BPA experts to question the "usefulness" of smaller sized bloodstains (l<3mm) as they may potentially be an overall better fit in regards to their shape and location on a particular spatter bearing surface encountered at a crime scene.

The ANOVA variance test used in this experiment found that there was no statistically significant difference between experimental (small and large stains) and actual values for the X (p-value=0.906), Y (p-value=0.262) and Z (p-value=0.688) co-ordinates. Furthermore, paired t-tests found that no statistically significant difference existed between use of small bloodstains (l<3mm) or large bloodstains (l>3mm) in determination of the experimental X (p-value=0.906), Y (p-value=0.262) and Z (p-value=0.688) values.

de Bruin et al., (2011) found that bloodstains with larger surface areas corresponded to less deviation in AOO determinations and it was suggested that a bloodstain width> 1.5mm gave a greater correlation to actual AOO values; small bloodstain widths in this study ranged from 0.61mm-1.81mm and did not show specific deviation from AOO at widths of <1.5mm. A proposed reason for bloodstains with smaller surface areas having less accurate results was *'issues distinguishing edges of the bloodstains, leading to errors in width and length measurements and subsequently impact angle errors'* (de Bruin et al., 2011). In this study the calculated average X co-ordinate values (small and large stains) showed minimal displacement from actual X values, however, the smaller bloodstains did show an overall smaller displacement (-0.21cm) to the larger stains (-0.32cm). Statistically- according to ANOVA (Table 4) and a paired t-test (Table 5)- there was no significant difference between the small and large stains when compared to the true X co-ordinate value. The experiment was however in agreement with research indicating X values were primarily underestimated in AOO determinations be it the small or large stains in this study *ie.* closer to the spatter bearing surface (Adam, 2012, Connolly et al., 2012, Kettner et al., 2015).

Interestingly, Reynolds et al., (2009) stated small droplets (l >3mm) with impact angles <15° showed an increase in deviation from the actual AOO and an overestimation of X values in these particular stains. In this experiment there were no impact angles <15° so direct comparisons could not be drawn, however, bloodstains with impact angles <25°

were examined. Only four small droplets had an impact angle <25° and of these, two overestimated the X co-ordinate value and two underestimated it. Although the dataset was not large enough to draw definitive conclusions, the results did indicate more research on impact angles and small bloodstains was needed. In future, for the purpose of creating stains with smaller impact angles, the point of origin can be moved closer to the spatter bearing surface. However, droplet oscillation is a significant factor in these cases (especially large stains) as droplets that have not reached maximum oscillation decay may create stains with unpredictable shapes leading to incorrect AOO determinations (Raymond et al., 1996a).

The experimental Y co-ordinate displacement from actual AOO values for both the large and small bloodstains was only 2% and 3% respectively. Large bloodstains fared slightly better than the small droplets by a mere margin of 1%. Experimental Z values on the other hand showed the most displacement among the three co-ordinates but this was an expected result as tangent method calculations are based on an assumed straight-line blood droplet flight path where gravity is neglected and the parabolic arc of a blood droplet trajectory is disregarded resulting in an overestimation of height (Macdonell, 1971). The large and small droplets showed an overall 13% and 15% displacement from the actual Z value respectively. Both the large and small bloodstains showed no statistically significant difference to the actual Z value (p-value (large)=0.008, p-value(small)=0.013) but small bloodstains did show less of a statistically significant difference to actual Z values than large bloodstains. The difference between small and large bloodstains was again negligible and not statistically significant (p-value=0.688). This is an interesting outcome since, if there was no statistically significant difference between large and small stains you would also expect there to be negligible difference in the interplaying forces that led to the formation of each of these stains. Surface tension for example, plays a greater role as droplet size decreases (Reynolds et al., 2009, Attinger et al., 2013) but this was not reflected in this study.

Pace (2005) presented a similar argument to the paper by de Bruin et al., (2011), stating that choosing more elliptical blood droplets decreased the deviation in AOO calculations and formed the conclusion that for impact angles above 50° even a small measurement error made a large difference in subsequent calculations. Both articles used computer assisted methods to determine the AOO but manual methods for ellipse measurements. The dataset for impact angles was not controlled in this experiment and only 8 out of 200 impact angles were above 50°, seven came from pattern nine's large stains and one from pattern two's large stains. It was noted that 90% of pattern nine's stains with impact angles >50° also measured <50cm from leading edge of the bloodstain to the AOC. Of the droplets that had an impact angle <50° (3 out of 10 stains) 100% measured >100cm from leading edge of the bloodstain to the AOC. This is in agreement with papers by Raymond., (1995) and de Bruin et al., (2011) which both state a distance of >1m improves AOO determinations.

It was also interesting to note that those patterns with large displacements for X, Y and Z followed the same trend whether they were small or large stains (patterns 2, 6, 9 and 10 shown in Figures 19 and 20). This highlights that stain selection plays a huge role in subsequent analyses and demonstrates real world conditions in that not all spatter patterns encountered at a crime scene have perfect or optimal stains available for selection.

5. Conclusion

In recent years, technological advances have far superseded the forensic science and legal fields and more times than not scientific procedures and forensic practices are being questioned by the judicial system and intelligence agencies. In BPA, small bloodstains have not been considered "useful" in AOO determinations because of their supposed unpredictable behaviour and the unknown nature of the forces that act upon them. This study has demonstrated that small bloodstains (l <3mm) can be employed in AOO determinations when used in conjunction with computer assisted ellipse fitting programs such as *Microsoft® Office Excel 2003 Auto Shape* and digital photography. No statistically significant difference between the small bloodstains (l <3mm) and large bloodstains (3mm> l<8mm) was reported in the analysis of this data and small bloodstains did not consistently show overestimation in X co-ordinate values at impact angles of <25° as observed by Reynolds et al., (2009). This paper, along with the study by Reynolds et al., (2009) has highlighted the minimal research conducted in bloodstain selection criteria for AOO determinations and the research presented here is only one step in the right direction. Future investigation into smaller bloodstains in AOO determinations and effects of impact angles on X co-ordinate calculations must be conducted to give more weighting to the preliminary findings of this study as the research here is compelling but not exhaustive.

6. References

ADAM, C. D. 2012. Fundamental studies of bloodstain formation and characteristics. *Forensic Sci Int,* 219**,** 76-87.

ATTINGER, D., MOORE, C., DONALDSON, A., JAFARI, A. & STONE, H. A. 2013. Fluid dynamics topics in bloodstain pattern analysis: comparative review and research opportunities. *Forensic Sci Int,* 231**,** 375-96.

BALTHAZARD, V., PIEDELIEVRE, R., DESOILLE, H. & DEROBERT, L. 1939. Etude des Gouttes de Sang Projecte (Study of Projected Drops of Blood). *Ann Med Leg Criminol Police Sci Toxicol,* 19**,** 265-323.

BEVEL, T. & GARDNER, R. M. 2008. *Bloodstain pattern analysis: with an introduction to crime scene reconstruction* Boca Raton: FL, CRC Press/Taylor & Francis

BRODBECK, S. 2012. Introduction to Bloodstain Pattern Analysis *Journal for Police Science and Practice* 2**,** 51-57.

BUCK, U., KNEUBUEHL, B., NATHER, S., ALBERTINI, N., SCHMIDT, L. & THALI, M. 2011. 3D bloodstain pattern analysis: ballistic reconstruction of the trajectories of blood drops and determination of the centres of origin of the bloodstains. *Forensic Sci Int,* 206**,** 22-8.

CARTER, A. L. 2001. The Directional Analysis of Bloodstain Patterns- Theory and Experimental Validation *Canadian Society of Forensic Science* 34**,** 173-189.

CARTER, A. L., FORSYTHE-ERMAN, J., HAWKES, V., ILLES, M., LATURNUS, P. L., LEFEBVRE, G., STEWART, C. & YAMASHITA, A. B. 2006. Validation of the Backtrack™ suite of programs for bloodstain pattern analysis. *J Forensic Identification,* 56**,** 242-245.

CARTER, A. L., ILLES, M., MALONEY, K., YAMASHITA, A. B., ALLEN, B., BROWN, B., DAVIDSON, L, ELLIS, G., GALLANT, J., GRADKOWSKI, A., HIGNELL, J., JORY, S., LATURNUS, P. L., MOORE, C. C., PEMBROKE, R., RICHARD, A., SPENARD, R. & STEWART, C. 2005. Further validation of the Backtrack™ computer program for bloodstain pattern analysis-precision and accuracy. *IABPA News,* 3.

CONNOLLY, C., ILLES, M. & FRASER, J. 2012. Affect of impact angle variations on area of origin determination in bloodstain pattern analysis. *Forensic Sci Int,* 223**,** 233-40.

DE BRUIN, K. G., STOEL, R. D. & LIMBORGH, J. C. 2011. Improving the point of origin determination in bloodstain pattern analysis. *J Forensic Sci,* 56**,** 1476-82.

EVANS, S. 2015. *Effect of haematocrit value on area of origin determination for bloodstain pattern analysis.* Honours, Murdoch University.

ILLES, M. & BOUE, M. 2011. Investigation of a model for stain selection in bloodstain pattern analysis. *Canadian Society of Forensic Science,* 44**,** 1-12.

JAMES, S., KISH, P. & SUTTON, T. P. 2005. *Principles of bloodstain pattern analysis: theory and practice* Boca Raton, FL, CRC Press.

KABALIUK, N., JERMY, M. C., WILLIAMS, E., LABER, T. L. & TAYLOR, M. C. 2014. Experimental validation of a numerical model for predicting the trajectory of blood drops in typical crime scene conditions, including droplet deformation and breakup, with a study of the effect of indoor air currents and wind on typical spatter drop trajectories. *Forensic Sci Int,* 245**,** 107-20.

KETTNER, M., SCHMIDT, A., WINDGASSEN, M., SCHMIDT, P., WAGNER, C. & RAMSTHALER, F. 2015. Impact height and wall distance in bloodstain pattern analysis--what patterns of round bloodstains can tell us. *Int J Legal Med,* 129**,** 133-40.

MACDONELL, H. 1971. Flight Characteristics and Stain Patterns of Human Blood. *US Dept of Justice NIJ Pub***,** 77.

PACE, A. 2005. The relationship between errors in ellipse fitting and the increasing degree of error in angle of impact calculations. *IABPA News,* 3**,** 12-14.

PESCHEL, O., KUNZ, S. N., ROTHSCHILD, M. A. & MUTZEL, E. 2011. Blood stain pattern analysis. *Forensic Sci Med Pathol,* 7**,** 257-70.

PIOTROWSKI, E. 1895. Ueber Entstehung, Form, Richtung und Ausbreitung der Blutspuren nach Hiebwunden des Kopfes [On the formation, form, direction, and spreading of blood stains after blunt trauma to the head].

RAYMOND, M. A., SMITH, E. R. & LIESEGANG, J. 1996a. Oscillating blood droplets--implications for crime scene reconstruction. *Sci Justice,* 36**,** 161-71.

RAYMOND, M. A., SMITH, E. R. & LIESEGANG, J. 1996b. The physical properties of blood--forensic considerations. *Sci Justice,* 36**,** 153-60.

REYNOLDS, M. 2008. *Bloodstain Size, Shape and Formation; implications for the bloodstain pattern analyst.* Masters of Forensic Science/ Doctor of Philosophy, University of Western Australia.

REYNOLDS, M., RAYMOND, M. A. & DADOUR, I. 2009. The use of small Bloodstains in Blood Source Area of Origin Determinations. *Canadian Society of Forensic Science,* 42**,** 133-146.

WONDER, A. & YEZZO, G. M. 2015. *Bloodstain patterns: identification, interpretation and application,* Boston: MA, Elsevier/AP.