UNIVERSITY OF AMSTERDAM

Institute of Interdisciplinary Studies

Master Forensic Science

Research Projects 2018-2019

Research projects 2018-2019

The University of Amsterdam's (UvA) Master's programme in Forensic Science, offered by the Faculty of Science, is unique in the Netherlands. The programme distinguishes itself from most international Master's programmes in Forensic Science by building on a range of scientific disciplines, such as Chemistry, Computer Science, Life Sciences, Mathematics, Physics, and other exact sciences. The goal of the programme is to train good scientists, armed with forensic knowledge and skills.

A part of the curriculum is a six-month internship during which scientific research is executed that is relevant to the forensic field. This document gives an overview of the capabilities of our students and the many ways in which a research project can be conducted.

For more information please contact:
fs-iis-science@uva.nl

Title	Organisation	Student	Previous education
Criminalistics – Human Factors			
The Influence of Investigative Psychologists on the Recognition and Collection of Evidence by Forensic Examiners	Netherlands Forensic Institute (NFI)	Rosanne de Roo	Psychobiology
Digital Forensics			
Social Network Analysis applied to the Jew hunter collaboration network active in 1944	Anne Frank a Cold Case Diary project groep - Proditione Media	Nienke Filius	Dentistry
Clothes Classification on Surveillance Images Using Convolutional Neural Networks	NFI	Jette Korthals Altes	Psychobiology
Digital Forensics - Biometrics			
CSI-PEEQ: Walking behaviour as a tell-tale for investigator proficiency.	Technical University (TU) Delft	Marijke Faber	Chemical engineering
Forensic Biology – Human biological traces			
Body Fluid Identification Using Mass Spectrometry Based Proteomics	NFI	Alex Shirin	Biomedical Sciences
Optimization and Evaluation of the Performance of the Massive Parallel Sequencing of Human Bone Samples using the Illumina MiSeq FGxTM Forensic Genomics System in Missing Persons Applications	International Commission on Missing Persons (ICMP)	Dyon Doensen	Biology and Applied Medical Laboratory Technology
Evaluation of the ParaDNA Intelligence Assay for the benefit of Bloodstain Pattern Analysis	NFI	Vidushi Ghai	Human Genetics
DNA from counterfeits; Optimisation of recovery and quantification of fingerprints.	University of Applied Sciences Amsterdam	Merel Kok	Health Science
MPSPlex - A large, massively parallel sequencing SNP panel for the identification of missing persons	ICMP	Felix Bittner	Biotechnology - Major Forensic Science
The development of miniSTR8A as a method for rapid direct PCR	Florida International University	Dide Boelens	Pharmacy
Investigation of the behaviour of DNA mixtures assessed by Massively Parallel Sequencing	Institute of Environmental Science and Research Ltd. Auckland, New Zealand	Anne Harteveld	(Molecular) Biology
Optimization of massively parallel sequencing workflow of mitochondrial DNA for degraded samples and comparison of heteroplasmic variation between tissues	NFI	Sophie Smit	Forensic Biology and Medical Laboratory Research

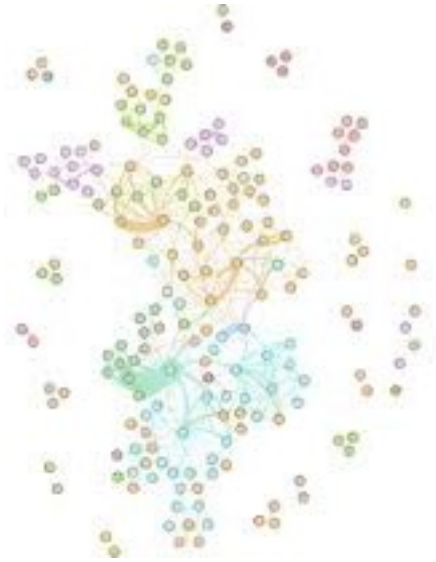
Title	Organisation	Student	Previous education
Forensic Biology – Non-human biological traces			
Bacterial endophytes and their role in the random contaminations of plant tissue cultures	University of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics	Mischa Tensen	Biology
Forensic Biophysics – Post Mortem Interval			
Estimation of the Post Mortem Interval Using Temperature-Based Methods:	GGD-Gelderland Midden	Haris Kalic	Pre-Med: Biology, Chemistry & Medical Science
Forensic Chemistry – Material Analysis			
Sniffing out genuine and counterfeit banknotes - HS-GC-MS analysis of euro bills	University of Applied Sciences Amsterdam	Anne van Vulpen	Molecular Science and Technology
Forensic Chemistry – Questioned Documents			
Differentiation between IBNS and non-IBNS ink stains on banknotes with hyperspectral imaging	Amsterdam University Medical Centre (AMC)	Dion van Kollenburg	Forensisch Laboratorium Onderzoek
Forensic Chemistry – Toxicology			
Developing a zebrafish embryo model for testing the addictive potential of substances in tobacco (smoke)	Rijksinstituut voor Volksgezondheid en Milieu (RIVM)	Romy Constant	Pharmaceutical Sciences
Forensic Medicine			
A closer look at injury reports: Use of injury reports: Current status and future recommendations	GGD Amsterdam	Puck de Jong	Biomedical Sciences
Study of the alterations of microvascularization in traumatic brain injury and its use in forensic medicine	University Autonoma de Barcelona, Morphological Sciences Department, Human Anatomy and Embryology Unit	Katrin Niedermaier	Biology
Forensic Medicine – Forensic Anthropology			
The prevalence of accessory sutures and wormian bones in a contemporary cohort of Dutch children	Amsterdam University Medical Centre (AMC)	Gisela de Heus	Biomedical sciences
Forensic Physics – Blood Pattern Analysis			
Determining the height of fall of blood droplets by scanning the volume of ‘dry’ drip stains with a 3D structured light scanner	NFI	Esther Snippe	Forensic Research
Forensic Statistics & Forensic Physics - Toolmarks			
Specific source vs. Common source reference scores to determine the evidential strength of Glock aperture shear marks	NFI	Ingemarie van Gilse	Medical Biology

Title	Organisation	Student	Previous education
Forensic Statistics & Forensic Chemistry - Microtraces			
A Pilot Study of Indirect Transfer and Persistence of Glass Fragments	NFI	Marouschka Vink	Natuur- en Sterrenkunde
Forensic Statistics & Forensic Physics			
Lethal smothering with a pillow – how 181 music festival visitors try to kill a dummy	TU Delft	Danique Prinsen	Chemistry (Forensics)

Criminalistics – Human Factors

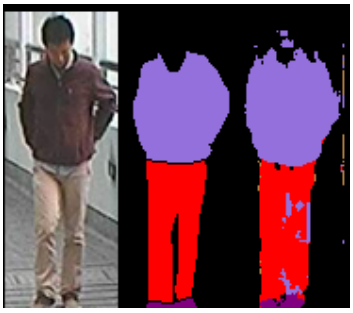
Student	Rosanne de Roo
Research carried out at	Netherlands Forensic Institute (NFI)
Supervisor	M. de Gruijter, PhD, Scientific researcher, Prof. dr. C.J. de Poot, Professor of criminalistics
Title thesis	The Influence of Investigative Psychologists on the Recognition and Collection of Evidence by Forensic Examiners
Abstract	Observations at a crime scene may usually be attributed to multiple explanations, making the recognition and selection of relevant traces extremely difficult. Therefore, knowledge from different disciplines should be integrated into crime scene investigation to improve the effectiveness of the investigation. Forensic science and criminology are often seen as two different disciplines, however, a new mindset is emerging with the proposition that the two disciplines ought to be bridged. Incorporating psychological theories on behaviour in forensic investigations is emerging around the world. As a result, direct involvement of psychologists at crime scenes is also arising. Investigative psychologists base their observations and interpretations of a crime scene on a behavioural perspective. However, the effect of psychologists involvement at the scene is unknown. The aim of this study is to investigate whether forensic examiners would detect deviant (behavioural) patterns in a death case at a home by themselves, or, whether they would possibly detect these patterns only after hearing the advice of an investigative psychologist. Therefore, the primary research question of this paper is: *"How does the advice of an investigative psychologist influence the decisions of a forensic examiner during the investigation of a deceased person in a home?".* With the sub question; how well are investigative psychologists able to recognize deviant (behavioural) patterns in a case with a deceased person in a home, compared to forensic examiners? In this study 40 forensic examiners and 14 investigative psychologists investigated a virtual 3D mock crime scene on a computer. The results of this study are confidential, however, provide valuable information that can contribute in bridging the forensic and criminological discipline by learning from each other's expertise.

Digital Forensics



Example visual representation Social Network Analysis

Student	Nienke Filius
Research carried out at	Proditione Investigative Media
Supervisor	Dr. M. Koeman M.Sc.
Title thesis	Social Network Analysis applied to the Jew hunter collaboration network active in 1944
Abstract	What led to the arrest of Anne Frank and the others in hiding at Prinsengracht 263 Amsterdam on 4 August 1944? One of the few established facts regarding the arrest, is that the arrest was led by SS-Hauptscharfürer K.J. Silberbauer in the company of at least three Dutch Sicherheitsdienst (SD) policemen. The actual number of Dutch SD policemen varies from three to eight by the different witness accounts. The SD policemen that were never identified might have known more about the circumstances that led to the arrest at Prinsengracht 263 Amsterdam. The aim of this research project was to investigate if a social network analysis, constructed from the collaborative ties between SD policemen and other Jew hunters who worked together on arrests in 1944, could aid in the search for these potential witnesses.

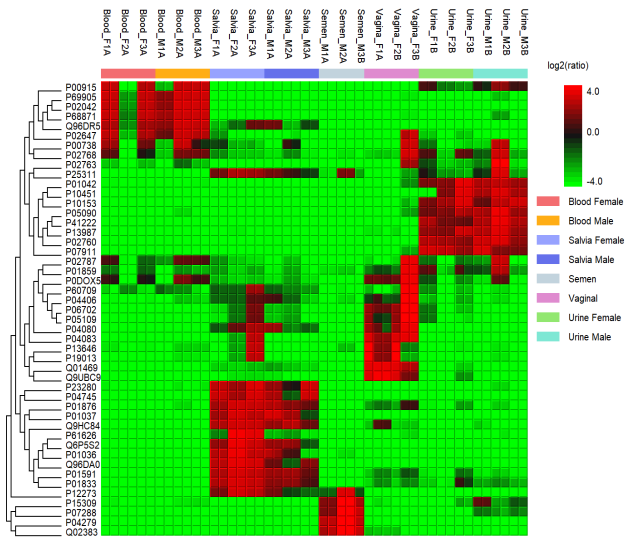


Student	Jette Korthals Altes
Research carried out at	Nederlands Forensisch Instituut
Supervisor	Andrea Macarulla Rodriguez
Title thesis	Clothes Classification on Surveillance Images Using Convolutional Neural Networks
Abstract	The aim of this research is the classification of clothing items on surveillance images in order to track down people. This can be done by using convolutional neural networks. Unfortunately, these convolutional neural networks need a lot of data to learn from, and datasets consisting of surveillance images with annotated clothing items are not publicly available. This explains the limited research that has been conducted on this topic. Two different approaches have been taken to train a convolutional neural network with a small dataset of surveillance images: a semi-supervised learning approach, and a transfer learning approach. A small subset of an existing surveillance dataset that is publicly available has been annotated for clothing items. For the semi-supervised learning approach, a Semi-supervised Generative Adversarial Network (SGAN) was used. The SGAN model has been trained on 25 images per class, 50 images per class, and 100 images per class. The highest accuracy was obtained when training on 100 images per class. This was 64% over 10 classes of clothing items. The baseline, a model that did not use semi-supervised learning, obtained an accuracy of 68% when trained on the same number of images . For the transfer learning approach, a semantic segmentation model was trained on a fashion dataset and tested on a surveillance dataset. A pixel accuracy of 60% was obtained versus 81% when the network was trained on the surveillance dataset. Of the number of instances of clothing items present in the images, 13% was correctly classified when the network was trained on the fashion dataset versus 95% when the network was trained on the surveillance dataset. The current method of semi-supervised learning does not outperform supervised learning on small datasets. The performance of the model trained on a fashion dataset could not compete with that of a model trained on a surveillance dataset. However, it is possible to classify clothing items on surveillance images, even using a fine grained method like semantic segmentation.

Digital Forensics - Biometrics

Student	Marijke Faber
Research carried out at	Technische Universiteit Delft
Supervisor	Dr. Arjo Loeve
Title thesis	CSI-PEEQ: Walking behaviour as a tell-tale for investigator proficiency.
Abstract	The results of the analysis of the traces found at the crime scene play a very important role in the investigation, reconstruction and as evidence of a crime. Crime scene investigators of the police have the task to find the traces. In this research a first insight in the correlation between the path length travelled, investigation time spent and other walking characteristics and the found traces by the crime scene investigator when investigating the crime scene, and if this correlation could relate to the efficiency, efficacy and quality of crime scene investigation is established. A MATLAB GUI was created for the analysis of the walking path of the crime scene investigators and a protocol was created and used to find the walk pattern manually. The walk pattern was plotted in the floorplan of the mock crime scene and the total path length and investigation time were calculated. In the walk patterns of the participants no clear differences in the patterns could be seen. During the path analysis it was noticed that there are different investigation approaches between participants. First a distinction is made between the stepwise approach and iterative approach. Furthermore, a separation could be made in the location of workstation, on the crime scene or outside the crime scene at the evidence table. The total path length was plotted against the following investigation parameters: total investigation time, number of secured traces, number of examined traces, percentage crime related traces, percentage examined traces, number of photos taken and total amount of traces. During plotting a distinction was made between the stepwise approach and iterative approach and separately between the different workstation locations. The results showed that using the stepwise approach a longer path length resulted in more (crime related) traces examined, as where by the iterative approach the opposite applies. This result had an influence on the efficacy and efficiency of the crime scene investigation Overall could be concluded that the stepwise approach probably results in a crime scene investigation with a higher efficacy, but the efficiency decreases when the path length increases.. Further research is necessary to determine if this relation holds up with a bigger dataset.

Forensic Biology – Human biological traces

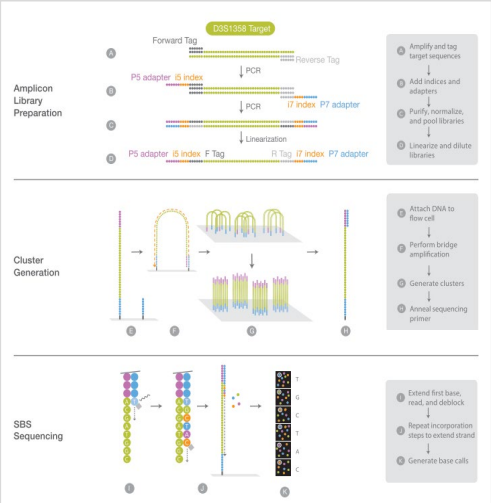


The heat map generated was used as a visualisation tool to represent the most abundant biomarkers present in the different body fluids. This data was generated using the LC-QToF

Student	**Shirin Alex**
Research carried out at	Netherlands Forensic Institute (NFI)
Supervisor	Dr. Marcel de Puit
Title thesis	Body Fluid Identification Using Mass Spectrometry based Proteomics
Abstract	In a forensic investigation, the identification of a biological matrix from complex mixtures or surfaces without the destruction of DNA becomes of utmost importance. Alternative methods support the reconstruction of crime scene events and steer the investigation towards the right track and also provides added evidential value in casework. Here, we established a protein extraction protocol and performed mass spectrometry-based proteomics to identify unique protein biomarkers for unambiguous identification of most commonly encountered body fluids at a crime scene. Thereby, in collaboration with the proteomics group at TU Delft, we identified a total of 18 high abundant unique protein biomarkers for the body fluids namely saliva, urine, blood, semen, and vaginal secretions.

ICMP (2019), Recovering human skeletal remains

Student	Dyon Doensen
Research carried out at	International Commission on Missing Persons (ICMP)
Supervisor	Dr. Thomas Parsons
Title thesis	Optimization and Evaluation of the Performance of the Massive Parallel Sequencing of Human Bone Samples using the Illumina MiSeq FGxTM Forensic Genomics System in Missing Persons Applications
Abstract	Over the year, millions of individuals disappear or have gone missing due to conflicts, human right abuses, disasters, refugee migration and/or organized crimes. Many families are left behind, desperate to know the fate of their missing. One of the key activities of the International Commission on Missing Persons (ICMP) is to provide technical assistance in identifying the missing individuals by conducting high-throughput DNA identifications through DNA typing and DNA matching with recovered human remains. The DNA matching most often involves kinship matching using the reference DNA profiles from family members in order to identify familial relationships. Insufficient reference samples and the heavily degraded and/or inhibited state of received skeletal samples can complicate the DNA analysis and might result in unidentified remains. With the introduction of Massive Parallel Sequencing (MPS) in the field of human identification it is now possible to simultaneously sequence short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs) in one single reaction with the purpose of retrieving more genetic information and increasing the discrimination power from even highly degraded samples. One of the commercially available MPS assays that has been developed specifically for forensic use is the Illumina MiSeq FGx™ Forensic Genomics System. This system, which includes the ForenSeq™ DNA Signature Prep Kit for library preparation, allows the targeted amplification and sequencing of 56 STRs and 94 identity informative SNPs (iiSNPs), with the option to include an additional 22 phenotypic informative SNPs (piSNPs) and 56 biogeographical ancestry SNPs (aiSNPs). Due to the overlap of the included aSTRs with the STRs being analyzed with traditional STR kits using CE technology, backward compatibility



Illumina Inc. (2015), Targeted Next-Generation Sequencing for Forensic Genomics

with currently existing databases is achieved.

The ICMP DNA laboratory has started the performance testing of the Illumina MiSeq FGx™ Forensic Genomics System for the targeted massive parallel sequencing of (degraded) human bone samples encountered in missing persons casework. Initial experiments have demonstrated that the standard library preparation protocol of the ForenSeq™ DNA Signature Prep Kit is not optimal for the processing of bone samples. The presence of inhibitors within the bone samples seem to make it all the way through sequencing and are most likely affecting the sequencing performance. The work reported here represents an attempt to purify the samples and remove inhibitors to optimize sequencing results, an extract purification step, new PCR1 buffer system and change of wash buffer (70% EtOH:TE) in the library purification have been incorporated in the procedure and applied to a set of 30 bone samples. Results have demonstrated the significant improvement in performance compared to the standard protocol, but further optimization of the protocol is recommended before validation of the modified ForenSeq™ DNA Signature Prep Kit for processing bone samples. However, the current state of the procedure enables the simultaneous analysis of autosomal, X- and Y-STRs and a panel of 94 iSNPs in one multiplex and therefore shows potential for increasing the operational efficiency in the laboratory. More important, it has shown to produce an increased amount of genetic information that can contribute to the kinship analysis in missing persons applications. With the potential to detect and confirm more distant relationships with a higher certainty it is worth to further optimize the procedure so it can ultimately be integrated as standard operating procedure at the ICMP.

Figure 1. ParaDNA Intelligence assay; Field portable instrument. The ParaDNA benchtop machine which has 4 in-built chambers containing the thermal cyclers, a user display and multiple attachment ports. The displayed user interface is used to log in to the ParaDNA system software and offers track and trace facility. The results of the ParaDNA runs are displayed on the screen as well along with the other functions

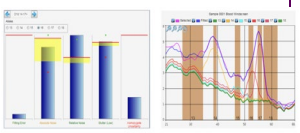
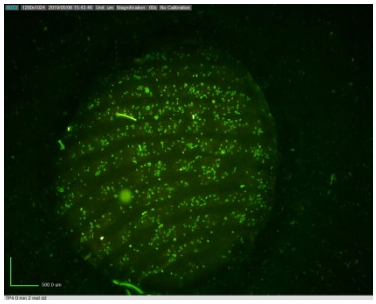
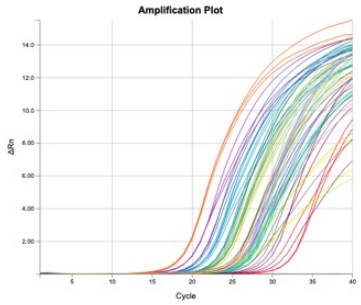


Figure 2. Image of the melt data panel as seen in the ParaDNA Data analysis software Bar graph on the left represents the ParaDNA results of the sample against the threshold values of specific characteristics. The graph on the right is the Melt graph. *Source: ParaDNA_User Guide_Data Analysis Software*

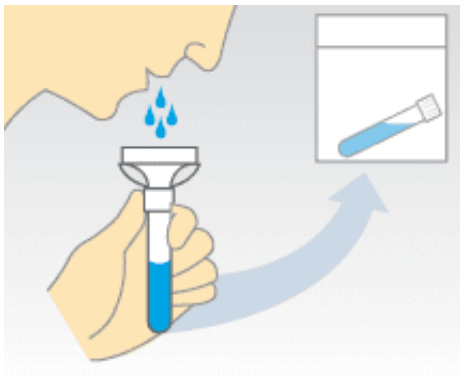
Student	**Vidushi Ghai**
Research carried out at	*The Netherlands Forensic Institute (NFI)*
Supervisor	*Matthijs Zuidberg*
Title thesis	*Evaluation of the ParaDNA Intelligence Assay for the benefit of Bloodstain Pattern Analysis*
Abstract	Rapid and mobile DNA technologies have gained quick interest in the forensic community. Many of these mobile DNA technologies are made with an intent to simplify and shorten the full range STR analysis procedure to provide rapid insight to the Scene of Crime officers (SoCOs). ParaDNA Intelligence assay is one such rapid DNA-STR typing technology. It is a benchtop instrument which can be used in lab or on-field environments. It utilizes direct PCR and fluorescent HyBeacons technology to genotype 5 STR loci plus Amelogenin (gender prediction). ParaDNA intelligence test apparatus consists of the portable ParaDNA instrument with 4 independent thermal cyclers that renders users the ability to run four different samples in parallel. It also utilizes an innovative 4-nib sample collector and a ready-to-use cartridge plate filled with PCR reagents. The easy to use machinery requires no prior sample preparation and provides genotyping results within 75 minutes. This report describes the internal lab validation of the ParaDNA Intelligence system for the benefit of Bloodstain Pattern Analysis (BPA) as carried out at the Netherlands Forensic Institute (The Hague, NL). The validation was performed in accordance with the internal validation guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDAM). Using ParaDNA Intelligence system can aid the BPA experts to gain rapid preliminary insights, improve the decision-making process, better the trace selection and help them in intelligent prioritization of the samples. Mock evidence samples were prepared by spiking known volumes of blood on a range of substrates. The data collected from these samples were used to evaluate the performance of the ParaDNA system. Performance characteristics that were tested included sensitivity, accuracy, repeatability, reproducibility, mixture studies and robustness. Results from this study demonstrate that ParaDNA Intelligence test can be effectively used for analyzing high volume bloodstains. All large bloodstain samples were genotyped successfully by the ParaDNA and passing criteria for all performance characteristics was achieved. However, it was noted that caution should be observed while working with low quantity and low-quality stains. A further field validation is recommended to assess the effect of external environmental factors on the ParaDNA Intelligence assay before it is implemented for actual field-work.





Student	Merel Kok
Research carried out at	Hogeschool van Amsterdam (HvA)
Supervisor	Drs. Jeanine Joling
Title thesis	DNA from counterfeits; Optimisation of recovery and quantification of fingerprints.
Abstract	DNA is the most powerful tool for identification in the forensic community and it is increasingly used in new forensic areas. One of these new areas is counterfeiting. Counterfeiters make use of two-layered paper to mimic the security features. In between the two layers, DNA from the counterfeiter can be present. The main goal of this research is to determine the possibility to recover DNA from counterfeits. Therefore, an optimal method to recover and quantify DNA out of fingerprints is investigated. Different master mixes and collection methods were tested and compared to each other. After selection of the best method, fingerprints from different paper types and counterfeits were recovered. The method that uses the 4N6 FLOQ® swab moisturised with Triton-X to collect the DNA, extract the DNA with the use of the NucleoSpin® DNA Forensic Kit and quantify the DNA using Gotaq® qPCR master mix and an Alu Ya5 primer set provided the best result. With this method, it was possible to recover around 70 pg/µl DNA out of one fingerprint from a glass slide. Another promising method used the l'Avely swab to collect the DNA in combination with Prep-n-GoTM as an extraction method. This method recovered around 10 pg/µl DNA out of one fingerprint from a glass slide. Both methods were tested for the recovering of DNA from thin papers and a real counterfeit. The best results were obtained with the 4N6 FLOQ® swab combined with the NucleoSpin® DNA Forensic Kit. This method recovered around 17 pg/µl DNA from a paper that had a smooth surface and around 3,5 pg/µl from 'fakeproof' paper, a paper that is known to be used to make counterfeits. From the real counterfeit around 3 pg/µl DNA was recovered. These quantities of recovered DNA can be enough to obtain a full profile. Further research will have to show whether it is actually possible to create DNA profiles and if the results are also applicable in real counterfeit crimes without controlled circumstances.

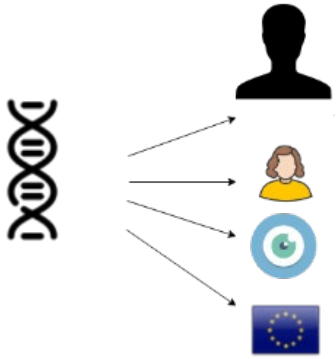
Student	Felix Bittner
Research carried out at	International Commission on Missing Persons
Supervisor	Thomas Parsons
Title thesis	MPSPlex - A large, massively parallel sequencing SNP panel for the identification of missing persons
Abstract	Missing persons are often identified by short tandem repeat analysis. Due to lack of reliable antemortem samples, DNA of unknown persons must be statistically compared to putative family members. When the DNA is severely degraded, locus dropout leads to a lower statistical power of the analysis. This alone can prevent identification, but is further compounded if only distant relatives are available. Single nucleotide polymorphisms can use shorter amplicons and are thus less susceptible to degradation, they however lack statistical power compared to STRs. To take advantage of this, ICMP is developing MPSPlex: A platform agnostic, massively parallel sequencing SNP panel with 1456 loci, designed for the identification of missing persons. This study identified 490 SNP loci in MPSplex with indicators of poor performance and suggested primer redesign approaches for 414 of them. For 153, primers could be redesigned, which lead to an average 10x coverage success rate of 0.99 with these loci on high quality DNA . After 55 loci were discarded during manual review and 61 loci were excluded at analysis, MPSplex reached an average 10x coverage between 0.95-0.99, depending on input. Finally, MPSplex's fitness for purpose was demonstrated by sequencing of 4 degraded bone samples. Here, 10x coverage rates and concordance compared favourably to co-sequenced NA12878 control DNA. These results establish MPSplex as a robust panel with over 1205 SNPs and 46 microhaplotypes for the identification of missing persons via degraded bone samples, even if only distant relatives are available. Further work should focus on robotic automation, optimisation and validation.



Methods used to collect saliva for direct PCR.

Student	Dide Boelens
Research carried out at	Florida International University
Supervisor	Dr. B.R. McCord
Title thesis	The development of miniSTR8A as a method for rapid direct PCR
Abstract	Forensic genotyping following the standard method involves multiple steps: DNA isolation, quantification, amplification and separation by capillary electrophoresis (CE). This process takes 1 to 2 days. The amplification step, multiplex polymerase chain reaction (PCR) of short tandem repeat (STR) markers, can take up to 2.5 hours. However, there are situations in which it would be very valuable to have a presumptive DNA profile within 20 minutes or less; for example in mass disasters, at border crossings or airport security. To do this, rapid direct PCR protocols have been developed with faster amplification times. Furthermore, direct PCR saves time because there is no DNA extraction and quantification. To accomplish an even faster PCR procedure, a set of reduced size STRs have been multiplexed and amplified; we call this kit miniSTR8A. This paper describes the development of a rapid direct PCR protocol using the novel multiplex MiniSTR8A. This method shows great potential to be used as a quick screening method to obtain a rapid presumptive DNA profile in situations in which time is limited.

MiSeq FGx™ Sequencer



Information that can potentially be obtained when a DNA sample is sequenced with a forensic MPS panel.

Student	**Anne Harteveld**
Research carried out at	Institute of Environmental Science and Research Ltd. Auckland, New Zealand
Supervisor	Dr. SallyAnn Harbison
Title thesis	Investigation of the behaviour of DNA mixtures assessed by Massively Parallel Sequencing
Abstract	Massively parallel sequencing is fast emerging as an increasingly useful tool for forensic science. It allows for simultaneous characterization of a DNA sample for autosomal-, X- and Y-STRs and identitity-, phenotypic- and ancestry-informative SNPs. As the final steps of the validation of this technology, a DNA mixture study was carried out with up to 4 known contributors to test the performance of the system and its ability to generate informative profiles. The aims were to compare the performance of MPS against standard casework protocols using capillary electrophoresis (PCR CE) and to determine the limit of detection of male DNA in a female background compared to Y STR DNA profiling. The ForenSeq™ DNA Signature Prep Kit and a MiSeq FGx™ Sequencer were used for this purpose. The study shows that minor components of 1% can be detected using the ForenSeq™ DNA Signature Prep Kit in up to 4 person mixtures. Overall Identifiler® (PCR CE) and the ForenSeq™ DNA Signature Prep Kit appear to achieve similar sensitivity limits, although MPS provides more information at a similar sensitivity level. With Identifiler®, minor male components were difficult to identify when in extreme mixture ratios with female major contributors but this was easier with MPS. Until probabilistic genotyping software for MPS mixtures is available, a binary method is applied to interpret MPS mixtures and in many cases is limited to 2 person mixtures or higher order mixtures with a very small minor third (or fourth) contributor. Further work to extend this study to more combinations and ratios and to apply laboratory determined stutter thresholds is in progress.

Student	Sophie Smit
Research carried out at	NFI
Supervisor	Ing. Kristiaan J van der Gaag
Title thesis	Comparison of Heteroplasmic Variation Between Buccal Cells and Hairs Using Massively Parallel Sequencing
Abstract	If it is a challenge to successfully extract a DNA profile using nuclear DNA, it may be possible through mitochondrial DNA. Mitochondrial DNA represents a lineage marker and an important characteristic of mtDNA is the occurrence of heteroplasmy. Heteroplasmy is the presence of more than one mtDNA type in an individual. One of the major challenges for interpretation of heteroplasmy is that the frequency of heteroplasmic variants can vary across different tissues, such as buccal cells and hairs. To observe and analyse heteroplasmy in this study, massively parallel sequencing (MPS) is being used. With MPS the detection limit for calling mixed variants is close to 3%, whereas the detection limit for Sanger sequencing is approximately 20%. This study will focus on heteroplasmic variation in the mtDNA control region for buccal cells and hairs (of the same individual) analysed with MPS compared to Sanger sequencing. The study is in collaboration with the NICC Brussel. It is necessary to provide recommendations for the interpretation of MPS mtDNA data in forensic casework. By analysing and comparing buccal references and corresponding hairs of the same individuals, we can potentially link low-level heteroplasmy between buccals and hairs. A total of 475 hairs and 26 buccal refs were analysed with MPS at the NFI. The same buccal refs and a selection of the hairs were analysed with Sanger sequencing at the NICC Brussel. Mismatches between buccal refs and hairs were observed when analysing the results with Sanger sequencing. However, by using the MPS allele calling threshold of 3%, mismatches were explained by low-level heteroplasmy in the buccals. The majority of the observed heteroplasmic sites in buccals were found in the hairs. High percentage heteroplasmic sites in hairs not occurring in the buccal references were rarely observed. Heteroplasmy was regularly observed in a low percentage of reads when analysing with MPS. However, more than two non-ref heteroplasmic sites in one hair were rarely observed. Recommendations were written for interpretation of MPS data from hairs specifically focussing on heteroplasmy.

Forensic Biology – Non-human biological traces

Student	Mischa Tensen
Research carried out at	University of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics
Supervisor	dr. Hans Breeuwer (IBED)
Title thesis	Bacterial endophytes and their role in the random contaminations of plant tissue cultures
Abstract	Many microbial organisms live inside plants (called endophytes) and can play a role in, e.g., plant growth. However, there is a lack of knowledge of the interactions between endophytes and their host plants. Besides, it might be the case that plants need certain endophytes to be able to live. Currently, in practice, propagation of ornamental plants through plant tissue cultures is widely used. In this process, the plant tissue cultures are sterilised to avoid pathogens. This might disturb the endophyte communities and might get rid of endophytes that are beneficial or essential for the plant. Which could be a reason for randomly occurring contaminations that happen in the propagation process. Furthermore, according to the European Union transport of plant material that is (visibly) contaminated or contains harmful bacteria is not allowed. Therefore, the random occurring contaminations pose a problem for the transport and legislation of plant tissue cultures. In this study we wanted to gain more insight into these contaminations. To do that the endophyte community compositions together with their functional profiles of Alstroemeria, Limonium and Phalaenopsis plant tissue cultures were investigated. The endophyte community compositions were investigated by 16s rRNA gene sequencing analysis and the functional profiles were predicted with Tax4Fun2. Furthermore, different media (Plant-based and nutrient-rich) were compared for the cultivation of endophytic bacteria. On average there was a lower amount of growth of endophytes on plant-based medium compared to LB medium and LB+Plant-based medium. The roots of Phalaenopsis plants showed more culturable endophytic growth compared to the leaves. Analysis of the endophyte community composition resulted in different community compositions per plant species and that the community composition does not differ immensely between healthy and contaminated plant tissue cultures. The most common genera over all the plant tissue cultures were Escherichia/Shigella, Hyphomicrobium, Luteibacter, Mycobacterium, Pelomonas and Roseomonas. The predicted functional profiles between healthy and contaminated communities were similar with only small differences in the Phalaenopsis endophyte communities. Because the healthy and contaminated endophyte communities are similar it is likely that the contamination was caused by normally occurring endophytes instead of external contamination. Therefore, we suggest that to gain further insight into how and why the contaminations occur the endophyte communities (composition and transcriptome) need to be investigated with a time series instead of investigating at a single time point.

Forensic BioPhysics - Post Mortem Interval

Student	Haris Kalić
Research carried out at	GGD Gelderland-Midden
Supervisor	Jacquo van Remmen
Title thesis	Estimation of the Post Mortem Interval Using Temperature-Based Methods: Evaluation of the Method used in the Netherlands and Development of a New Protocol
Abstract	Background. Forensic physicians (FPHs) in the Netherlands are tasked with the responsibility of discriminating between suspicious and inconspicuous deaths. However, they are limited in their means to do so, as their examination is exclusively external. The estimation of the post mortem interval (PMI), which is crucial to their post mortem examination (PME), is particularly difficult. Methods considered current best practice have varying accuracy reported in literature and result in large intervals spanning many hours. The aim of this study was to identify weaknesses in the PMI estimation by FPHs in the Netherlands and to implement a new protocol in an attempt to improve it. Methods. A three-part study was performed. First, a questionnaire was spread among forensic medical professionals to assess their attitudes with regards to PMI estimation. Secondly, a database consisting of 1296 PMEs was consulted and used to assed the accuracy of estimations made using several different methods. Finally, a new protocol was developed and implemented. Using this protocol 78 PMEs were performed. Accuracy of the PMI estimation was assessed for these PMEs. Results. Estimations made using rigor and livor mortis from retrospective data achieved a 98% (n=448) and 96% (n=441) accuracy respectively. Correlation of estimated PMI by Henssge with true PMI was 0,651 (P<0,05, n=18) before implementation of the protocol and 0,879 (p=<0,001, n=15) after implementation. This increase was not significant ($z_{difference}$ = 0,126). Using the new protocol, accuracy of estimation by mechanical excitability of the biceps could be assessed in 8 cases. 5 of these were accurate, while for the remaining 3 accuracy remained unclear. No incorrect estimations were made using this method. Conclusion. A weakness of the current PMI estimation in the Netherlands is its low level of standardization and consistent application. As such, there is room for improvement in FPHs familiarity with the method, in particular with regards to the corrective factor estimation. Although the difference was not significant, implementation of the protocol resulted in a higher achieved accuracy using the Henssge method. This suggests that putting a greater emphasis on routine application of the method may improve its reliability in practice.

Forensic Chemistry – Material Analysis

Student	Anne van Vulpen
Research carried out at	Hogeschool van Amsterdam (HvA)
Supervisor	dr. Marc van Bochove
Title thesis	Sniffing out genuine and counterfeit banknotes - HS-GC-MS analysis of euro bills
Abstract	Raids on automated teller machines (ATMs) are currently used by criminals to quickly gain access to cash. As these raids cause cassettes wit IBNS indelible ink to explode, banknotes in these ATMs will become stained. Until today, there is no quick and efficient method to detect and remove these stained banknotes when in circulation. Research has shown several possibilities of using headspace gas chromatography with mass spectrometry (HS-GC-MS) profiling for the detection of volatile compounds in the headspace of genuine banknotes. In this study, a suitable headspace sampling method was developed, that can be used to characterize the headspace of Euro banknotes. Settings have been optimised for a reproducible, robust method for the sampling, gas chromatography and mass spectrometry. With these standard settings, the headspace of genuine banknotes and IBNS stained banknotes have been measured. Several peaks in the chromatogram were appointed to belong to specific compounds. A characteristic profile for the headspace of genuine banknotes as well for the headspace of IBNS ink was determined. The headspace of non-circulated, fresh banknotes were compared to the headspace of banknotes that were in circulation. Furthermore, the chromatograms of genuine banknotes and IBNS stained banknotes were compared, to find similarities and/or differences. Other inks visually similar to IBNS ink were measured and their headspace profiles were compared with the profile of IBNS inks, to determine potential false positives. In conclusion, the headspace profiles of IBNS ink and Euro banknotes are significantly different. However, regarding the headspace of banknotes stained with IBNS ink, the different components cannot all be directly appointed to their source. It is likely that components in IBNS ink affect the composition of the headspace profile of Euro banknotes. The components that have been appointed as originating from either IBNS ink or Euro banknotes could be used in the development of a device for real time detection of IBNS stains on Euro banknotes.

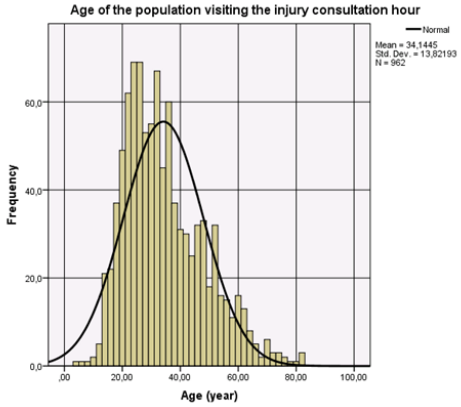
Forensic Chemistry – Questioned Documents

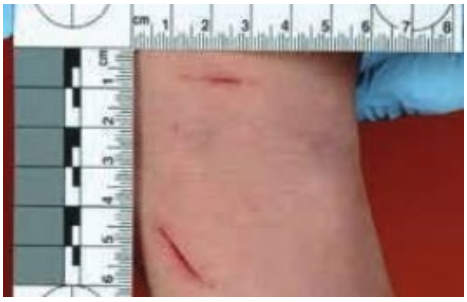
Student	Dion van Kollenburg
Research carried out at	Amsterdam UMC (AMC)
Supervisor	Prof. dr. Maurice Aalders
Title thesis	Differentiation between IBNS and non-IBNS ink stains on banknotes with hyperspectral imaging
Abstract	Automated teller machines (ATMs) are often raided by criminals. Though these crimes have recently been on the decline in the Netherlands, the border regions of Belgium and Germany have seen an increase over the last few years. Intelligent banknote neutralisation systems (IBNS) have been employed to invalidate the stolen money during the raid by spraying a special IBNS-ink on the banknotes. However, cash-in machines (CIMs) – of which there are plenty in the Netherlands – are not outfitted with a method to detect these inks. The result is that criminals are able to deposit their money without repercussions, leaving the Dutch national bank (De Nederlandsche Bank, DNB) with stained notes and no way to reclaim the stolen money. This study aims to solve this problem by using hyperspectral imaging (HSI) to determine whether the ink stains found on banknotes are IBNS ink or non-IBNS ink and as such take the first step to outfitting CIMs and the sorting machine at DNB with the tools needed to more strongly discourage ATM raid crimes. Initially, analyses have been performed on blank banknote paper in order to differentiate the inks without interference from the complex graphical structure of banknotes and determine the optimal parameters for the method. The blank paper was stained with different inks, both IBNS and non-IBNS, and imaged with a hyperspectral camera. The resulting spectra were processed using spectral angle mapping (SAM) to quantify the differences between spectra. Several processing parameters were tested, resulting in the best method for differentiation having been found to be the application of a standard normal variate (SNV) pre-processing over a 450-700nm wavelength range before applying SAM. The study was continued by identifying the different regions of interest (ROI's) on banknote edges where different substrate effects could affect the spectrum of the inks. The various inks were applied to these ROI's and imaged. Using the optimised method, the spectra of every ink at every ROI were processed and used to construct ROC-curves, which could be used to determine spectral angle cut-off values at each ROI for the exclusion of false negative or false positives as the circumstances require. It is recommended that false positives are minimised at CIMs and false negatives are minimised in the sorting process at DNB. To make the analysis faster, easier and cheaper for practical use at DNB, a trial with simulated multispectral imaging (MSI) was started to assess the theoretical reliability of MSI for differentiating between IBNS and non-IBNS inks. Five wavelength bands were used. The same processing methods as in HSI were applied. This resulted in a comparison of ROCcurves which showed the theoretical applicability of MSI in the detection of IBNS inks on banknotes. It is recommended to further research actual, non-simulated MSI.

Forensic Chemistry – Toxicology

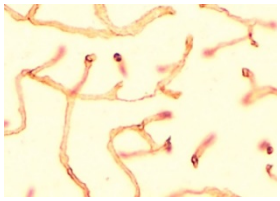
Student	Romy Constant
Research carried out at	National Institute for Public Health and the Environment (RIVM))
Supervisor	Dr. Anne Havermans
Title thesis	Developing a zebrafish embryo model for testing the addictive potential of substances in tobacco (smoke)
Abstract	Zebrafish are emerging as a promising model to study addiction to drugs of abuse. However, studies about tobacco cigarette addiction with the use of zebrafish are limited. Despite the fact that nicotine is the major addictive component in tobacco, other components in tobacco cigarettes may also contribute to tobacco addiction, for instance by mimicking or enhancing the addictive effect of nicotine. Further evaluation of tobacco components is essential for understanding their impact on tobacco addiction. In the here presented study, we developed a zebrafish embryo model, with the use of nicotine as a reference compound, for testing the addictive potency of components in tobacco (smoke). For this purpose, it was tested whether exposure to nicotine leads to tolerance in zebrafish embryos. In the current study, we found that zebrafish embryos that did not receive any treatment showed an increased activity after transition from light to dark, which is attributed to an increased stress/anxiety level in zebrafish embryos. This response of zebrafish embryos to transition from light to dark was suppressed after acute nicotine exposure. However, we found that prolonged exposure of nicotine during pre-treatments lead to tolerance and significantly attenuated the effect of subsequent acute nicotine exposure. The activity of nicotine pre-treated zebrafish embryos was returned to levels similar to zebrafish embryos that did not receive any treatment, indicated that pre-treatment with nicotine prevented the effects of acute nicotine. Such adaptation to nicotine may be due to several mechanisms, for example to neuroadaptations at the cellular or biochemistry levels. Further method validation is needed before the zebrafish embryo model can be a used to identify potential addictive substances in tobacco (smoke). If this zebrafish embryo model is applicable to investigate potentially addictive tobacco components, this model may also be useful for testing the addictive potency of drugs and medicines.

Forensic Medicine





Student	Puck de Jong
Research carried out at	Public Health Service (GGD)
Supervisor	Maartje Goudswaard
Title thesis	A closer look at injury reports
Abstract	Insufficient examination of the nature and cause of injury may lead to incorrect conclusions when it comes to fact-finding. Insufficient forensic medical expertise or inadequate injury reports has direct consequences in cases where a victim is injured. The need for high quality reporting in injury assessment is also emphasized by the public prosecutor's office in a letter to the ministry of Justice and Safety (November 8th 2018). The goal of this research is to investigate to what extend the injury reports made by the forensic medical department of the Public Health Services (PHS) Amsterdam are used during police investigations and legal cases and what they contribute. To investigate this, interviews are held with different individuals involved in the criminal proceedings. In addition, all data on injury reports made in 2016 are gathered and investigated. The goal of this data analysis is to provide more information on the characteristics of the cases and the specifics, such as quality of the injury reports.



Student	Katrin Niedermaier
Research carried out at	Universitat Autonoma de Barcelona
Supervisor	Maria Luisa Ortega Sanchez
Title thesis	Study of the alterations of microvascularization in traumatic brain injury and its use in forensic medicine
Abstract	The morbidity of cerebral trauma is determined by the appearance of secondary lesions: ischemia and endocranial hypertension. These lesions appear due to alterations of encephalic microvascularization. The main objective of this work is the study of the alterations that occur in encephalic microvascularization in traumatic brain injury (TBI). From the forensic point of view, the objective has been to assess the role that AQP4 plays as one of the possible markers in TBI, which could be of help in medical forensic studies when lesions are not evident. Samples of cerebral cortex of 5 deceased individuals without apparent CNS pathology (control group) and of 5 individuals deceased due to severe TBI (TBI group) have been studied. The study has been based on immunolabels for Collagen IV, type 4 aquaporins (AQP4) as well as neuronal Nissl-stains. The samples were visualized by optical microscopy. The results obtained showed that the study of the areas of injury and perilesion in the TBI group present macroscopic differences which do not correspond to the microscopic study, since there are no significant differences in neuronal or microvascular loss. These findings determine that in TBI microscopic studies should be performed since at macroscopic level lesions can go unnoticed. The TBI group has presented a decrease in neuron density, as well as a significant decrease in its vascular (capillary) density, both at level of lesion, perilesional focus and in areas at distance, a fact that we correlate with the appearance of secondary lesions such as cerebral edema and ischemia. We have observed that in the TBI group, capillaries show corrugation phenomena, as well as breakage of intervascular bridges (IBs). AQP4-staining has shown that the TBI group presents an activation of astrocyte feet at distance from the primary lesion, which was also activated early, in the first hours of trauma. This phenomenon was observed in an individual of the control group where death occurred through mechanical asphyxiation, with the consequent formation of cerebral ischemia and edema. Therefore, we correlate that the activation of AQP4 is not specific to TBI but that these are activated when cerebral edema is present. All the findings observed in the TBI group can be correlated with a disruption of the cerebral parenchyma angioarchitecture, which facilitates tissue ischemia. However, we cannot assure that the activation of AQP4 is a specific TBI-marker and, thus, its value in forensic medicine.

Forensic Medicine – Forensic Anthropology

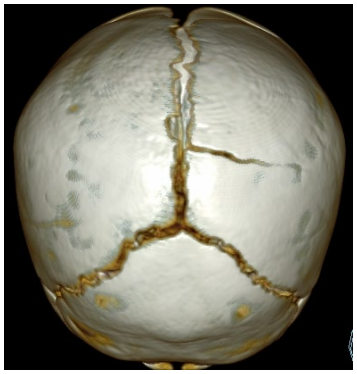


Figure 1 A case example of the difficulties in differentiating accessory sutures like this from fractures. In this case it was determined to be an accessory suture because of the absence of

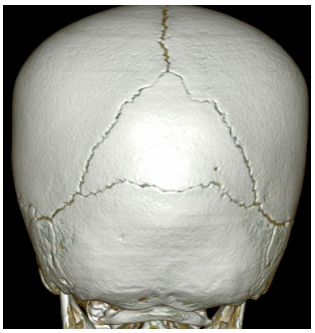
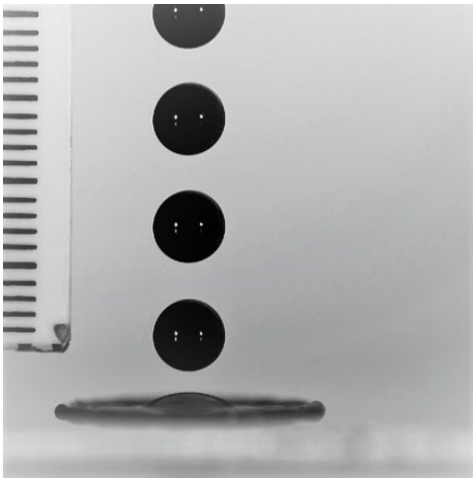


Figure 2 this figure shows a large triangular wormian bone at the location of lambda, which is also called an Inca bone.

Student	Gisela de Heus
Research carried out at	Amsterdam UMC
Supervisor	Prof. Dr. R.R. van Rijn
Title thesis	The prevalence of accessory sutures and wormian bones in a contemporary cohort of Dutch children
Abstract	Child abuse is a widely prevalent problem in the Western world. It is estimated that 118.000 to 180.000 children are abused in the Netherlands annually, of which physical abuse constitutes almost 18% of all abusive cases, with Abusive Head Trauma (AHT) being categorised as a severe form. In child abuse cases, skull fractures are a strong indication of AHT. However, the presence of accessory sutures and Wormian bones, sometimes lead to erroneous conclusions. This is because accessory sutures sometimes present similar morphologically characteristics on radiographs as fractures. For this research, 532 cranial CT scans of children below the age of 6 were selected. The 3D reconstructions of the scans were retrospectively reviewed by two observers, one of which an experienced paediatric radiologist. The presence of various accessory sutures and wormian bones were scored and evaluated. The results showed that accessory sutures were present in 34.3% of the cases. Of these accessory sutures, the metopic and mendosal suture were seen most frequently with 29.9% and 23.9% respectively. Other accessory sutures that were found including the intraparietal horizontal suture, intraparietal vertical suture, obelion suture and an occipital unossified midline were seen less frequently with 7.2%, 3.8%, 2.2% and 0.9% respectively. Most accessory sutures were found in the lower age groups and particularly the prevalence of the metopic and mendosal suture showed a decrease with age. Wormian bones were found in 78.3% of the cases, with the most frequent location being the lambdoid suture with 63.8%. Knowledge about the existence, prevalence and topographical location of various accessory sutures and Wormian bones will aid in the ability to make correct diagnosis and thereby increase the evidential value of the diagnosis in cases of abuse. However the results have to be interpreted and used with care.

Forensic Physics – Blood Pattern Analysis



Blood droplet impacting on a solid surface

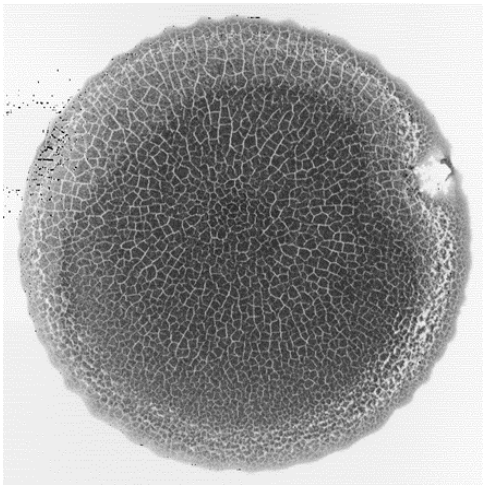


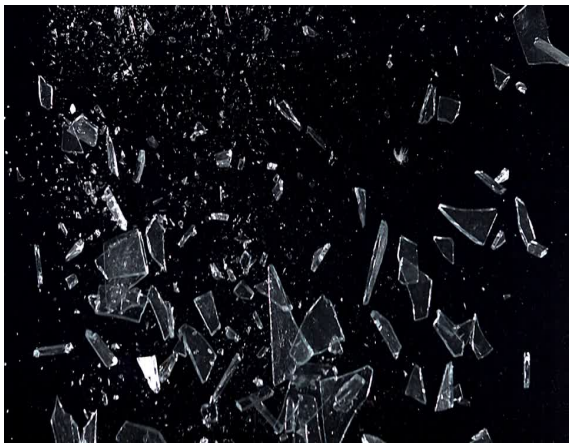
Image of a 'dry' drip stain produced with the 3D structured light scanner

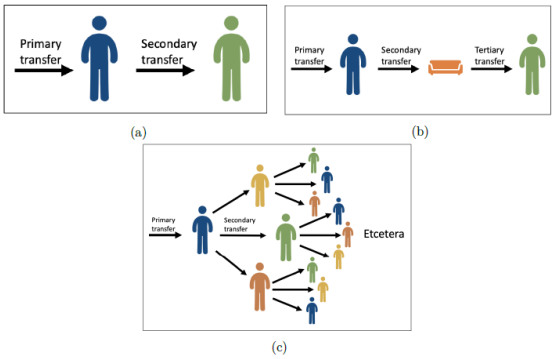
Student	**Esther Snippe**
Research carried out at	The Netherlands Forensic Institute (NFI)
Supervisor	Dr. G.J. Edelman, Ing. J.C.M. Limborgh
Title thesis	Determining the height of fall of blood droplets by scanning the volume of 'dry' drip stains with a 3D structured light scanner
Abstract	Forensic investigators discover bloodstains and bloodstain patterns at crime scenes on a regular basis. Although there are several methods to determine the area of origin from impact patterns, there is, despite several attempts, not yet a reliable method to determine the area of origin from bloodstains that resulted from falling blood droplets that formed due to gravity, the so called drip stains. Information about the height of fall of a blood droplet can be crucial in cases in which the position of a blood source during an impact is disputed. In this research project the diameter and volume of 'dry' drip stains were scanned with a 3D structured light scanner. With the help of the diameter and volume of the 'dry' drip stain, the drying ratio, spreading ratio and impact velocity, the height of fall of the blood droplet was calculated. This study shows that there is a positive linear relation between the height of fall of a blood droplet and the calculated height of fall based on the scanned diameter and volume of a 'dry' drip stain. There are, however, still some scientific challenges that need to be overcome before the technique can be implemented into casework.

Forensic Statistics & Forensic Physics - Toolmarks

Student	Ingemarie van Gilse
Research carried out at	NFI
Supervisor	Martin Baiker – Sorensen and Erwin Mattijssen
Title thesis	Specific source vs. Common source reference scores to determine the evidential strength of Glock aperture shear marks
Abstract	Introduction: Firearms are often involved in criminal cases with human casualties. In those cases, a comparison of firearm marksisimportant. Depending on what evidence is available, a common source (CS) or specific source (SS) database can be set up to analyze cartridge cases. These databases consist of same source and different source distributions, which can be set up with similarity scores. The CS and SS approaches both have their pros and cons, but they are often applied incorrectly in practice due to time restrictions and investments. Therefore, this study poses the following research question: "Does the evidential strength differ between the specific source vs. common source reference databases of Glock aperture shear mark evidence?" Methods: 20 Glock pistols were used, each of which fired 25 test shots with 9mm Luger Fiocchi cartridges. The cartridge cases were scanned with a 3D surface scanner. With scratch mark comparison software, the aperture shear marks were cropped. Similarity scores were calculated with Matlab to build 20 CS same source and 20 CS different source distributions and 500 SS same source and 500 SS different source distributions. The likelihood ratio (LR) was calculated between 0 and 1 with steps of 0.001 and presented in a log10(LR) gradient. LR ratios were calculated to compare the CS LRs with the SS LRs. Results: Visually, the CS distributions of the 20 firearms seem to correspond very well. The SS distributions seem to differ between the different firearms. Also, the LR gradients differ for the SS distributions. The LR ratios seem to differ between the weapons. For some firearms, the CS LR seems to be larger than the SS LR, while for other firearms the SS LR is larger than the CS LR. Conclusion: The same source and different source distributions of the CS and SS approaches differ from each other. This results in different evidential str

Forensic Statistics & Forensic Chemistry - Microtraces





Student	Marouschka Vink
Research carried out at	Netherlands Forensic Institute (NFI)
Supervisor	Prof. Dr. Marjan Sjerps
Title thesis	A Pilot Study of Indirect Transfer and Persistence of Glass Fragments
Abstract	The indirect transfer of glass is a rather unexplored field in forensic science. This pilot study identified relevant questions and designed three experiments to examine the indirect transfer, persistence and recovery of glass fragments. Three scenarios explored the indirect transfer of glass fragments onto fleece jackets and their persistence through hugs, through consecutive use of an office chair and through living in the same house. The results show that secondary transfer through hugs occurs frequently and that glass fragments remain on the garment even after 8 hours. Secondary transfer to the office chair was observed in 2 out of 8 repeats whereas only one repeat showed tertiary transfer onto the recipient fleece jacket. Furthermore, the results show that glass fragments are not easily indirectly transferred between housemates merely by being in the same room. A Bayesian network for a hypothetical case example showed that it is desirable to further investigate the indirect transfer and persistence of glass fragments through hugs in larger studies. This pilot study is suitable for a larger study on secondary transfer through hugs. However, the experimental set-up of the studies on the tertiary transfer through consecutive use of an office chair and on the indirect transfer between housemates should be modified before being executed on a larger scale.

Forensic Statistics & Forensic Physics

Student	Danique Prinsen
Research carried out at	TU Delft, Faculty of Mechanical, Maritime and Materials Engineering (3mE)
Supervisor	Dr. Arjo Loeve
Title thesis	Data analysis of Lowlands experiment; smothering to death using a pillow
Abstract	The criminal activity of smothering a person to death is very hard to confirm by forensic investigators because the crime scene often lacks on incriminating evidence. This study has investigated the activity of smothering to death using a pillow. Therefore 181 subjects were asked to pretend to smother a dummy to death using a pillow at Dutch music festival Lowlands. A questionaire was filled in by each subject concerning; gender, age, handedness, length, weight, alcohol use and drugs use. Forces applied to the dummy were measured and videorecordings were made of each individual. The goal was to identify the approaches of smothering along with analysing the variation of forces, with the intention to link these to the finger and hand markings that are left on the pillowcases. The questions arose if there were any volunteer characteristics or approaches of smothering that have an effect on the smothering force that is applied. The aim of the study was to gather information about how markings on the pillow are deposited, to help assesing the activity level evaluation in forensic science cases. The results showed a mean force of approximately 247 N, and a maximum force of 316 N applied by the subjects during smothering. The volunteer characterisics that were influencing the required duration time of smothering were length and drug use. The force a person applied was influenced by the alcohol intake. Three approaches of smothering happened to have an effect; the main position of the hands, body weight position and use of it, and the posture of the legs. For the position of the hands, a higher mean force and a shorter time is measured when pressure is excerted with both hands on the head, while both hands tightly next to head clearly has a lower force and longer smothering duration. The body weight position reaches the highest force and the shortest time when leaning with the chest above the dummy head. For the posture of the legs, the approach with the highest maximum force is the one with both feet on the ground, or both knees sitting on the bed. The forces measured and identified approaches can possibly in the future be linked to markings on the pillowcases.