



To: Institute of Chemistry
University of Silesia in Katowice

Genova, September 3rd 2021

Review Report on the PhD thesis: "Toward a spectroscopy-based approach for estimating time elapsed since bloodstains deposition – Development of a novel framework for blood evidence evaluation" by Ms. Alicja Menżyk MSc

1. Formal background

The official letter from the Institute of Chemistry of the University of Silesia in Katowice, signed by the Director, Professor Mirosław Chorążewski, and dated on the 1st of July 2021, designates me as a reviewer of the PhD thesis presented by Ms. Alicja Menżyk MSc in the same Institute.

The work was supervised by two internationally recognised specialists in the field of forensic analytical chemistry: Prof. Grzegorz Zadora, University of Silesia in Katowice (principal supervisor) and Dr. Eng. Agnieszka Martyna (assistant supervisor).

2. Introduction

The present research work addresses a key forensic issue in the evaluation of crime scenes: the estimation of time elapsed since the deposition of a bloodstain – a fundamental but still open question. The forensic and scientific backgrounds, as well as the ground-breaking objectives of the present thesis, are very clearly presented in the introduction chapters.

The analytical strategy proposed in the present PhD thesis is sound, as it is based on state-of-the-art techniques, both for data acquisition and for data processing. Namely, an analytical protocol based on Raman spectroscopy and likelihood ratio models has been developed, optimised, and validated. Results are critically discussed.

The innovative analytical solutions proposed as a frame for the present research work are of a great interest not only to the forensic sciences, since they provide the analytical scientists



and, in particular, the data analyst with effective and targeted strategies that can be effectively implemented also in different application sectors.

3. Work contents

The PhD dissertation entitled "Toward a spectroscopy-based approach for estimating time elapsed since bloodstains deposition – Development of a novel framework for blood evidence evaluation" consists of 202 pages, it is organised into 11 chapters and completed by an exhaustive bibliography (350 references), lists of abbreviations, symbols, figures, and tables, as well as two appendices containing further details on spectral processing and the Candidate's CV, respectively.

Chapters 1-3 introduce the forensic and scientific background of evidence dating in a very clear way, describing the state of the art and pointing out the objectives of the research work carried out and described in the dissertation.

Chapter 4 presents the biochemical mechanisms of ex vivo blood degradation, with a focus on the effect of external factors (especially, environmental ones) that are known to have an influence on the degradation processes.

Chapter 5 describes the physic-chemical principles of Raman spectroscopy and the basic constituents of the related instrumentation. Spectral features arising from vibrations involving haemoprotein bonds are also discussed. The choice of Raman spectroscopy is critically justified thorough a critical overview of results obtained by application of various spectroscopic techniques to address the issue of bloodstain dating during the last decade. All of the records examined are very clearly organised and efficiently presented in Table 5.1.

In Chapter 6, the computational strategies applied for the evaluation of the degree of bloodstain degradation (namely, likelihood ratio-based approaches) are presented, with a focus on the computation of model parameters and on the validation and assessment of performance.

Chapter 7 describes the chemometric strategies applied to minimise the amount of unwanted information embodied within the Raman signals: namely, different pre-processing transforms and an optimisation strategy (based on genetic algorithms) are presented.

Chapters 8-10 thoroughly describe the experimental plan and the results of the research work, with a distinct focus on the spectroscopy and on data analysis. Acquisition of Raman



spectra was carried out at the Chemistry Department of the University of Torino (Italy), during a research period spent by the Candidate as a frame for a scientific collaboration.

Chapter 11 presents a critical summary of the key achievements of the research work, from which clear lines for potential future developments in the bloodstain dating framework are traced.

Given the high quality that generally characterises the contents of the present PhD thesis, only a limited number of minor points and marginal remarks – listed below – can be raised. More than criticisms against the work presented, most of them can be taken as suggestions for further studies in the field.

- In the present document – and, particularly, within Chapter 7 – likelihood ratio approaches are presented in contraposition to chemometrics (see, for instance, the title of Chapter 7: “combining chemometrics and likelihood ratio framework for evaluating spectral data”). I do believe that such a divergence mainly reflects a potential divergence between academic communities, rather than a real opposition on a scientific basis. In fact, according to the definition given by the International Chemometrics Society (and partially reported, with a quotation from Svante Wold, in Chapter 1, page 5, of the present dissertation), chemometrics “uses mathematical and statistical methods [...] to provide maximum chemical information by analysing chemical data”. I think this definition fully embraces likelihood ratio approaches, when they are applied on chemical data.
- Implementing some tools derived from multivariate design of experiments (e.g., response surface methodologies) might have helped in lighting up the “profound obscurity” (see Paragraph 4.3.1, page 32) that still characterises the understanding of dependencies between the degradation processes of bloodstains and their initial composition, as well as the role of external factors. Some of the papers cited in the bibliography tried to follow such an approach, but only to a limited extent.
- A multivariate exploratory analysis performed on the sets of Raman spectra investigated (e.g., by principal component analysis – PCA) would have provided the reader with a comprehensive evaluation of spectral evolution over time. More targeted approaches, such as evolving factor analysis (EFA) and multivariate curve resolution (MCR), would have provided an in-depth and direct understanding of the



changes detectable in spectral features, linking them to the related changes in the chemical composition of samples investigated according to the degradation processes.

- Being a family of methods, genetic algorithms should be preferably referred to using the plural form (e.g, in the title of Paragraph 7.2).

4. Methodology, clarity of research and scientific correctness

The present doctoral thesis describes important results achieved in the field of forensic investigations by the development and application of innovative analytical strategies and advanced data processing tools for the determination of bloodstain ageing.

The introduction section and the bibliography are fully exhaustive and provide the reader with all the information required to understand the aim and the scientific/technical features of the research work that was carried out.

Analytical methods applied were properly and rigorously deployed in all of their stages, from sampling and sample pre-treatments to optimisation, and properly validated.

Instrumental signals were processed by appropriate techniques for the minimisation of unwanted contribution (pre-processing and optimisation) and for supervised data modelling. The models obtained proved to be potentially effective for a practical implementation in the field.

Results are fully convincing and clearly presented, with an adequate number and quality of tables and figures.

The whole document is very well structured and written in a rigorous and clear way, and might definitely serve as a template for PhD students to illustrate how to write a PhD thesis.

Most of the results presented and discussed therein have been published on qualified international scientific journals, confirming the relevance and the impact of the research carried out by the Candidate.

5. Conclusion

The present research work and the results described in the present PhD dissertation are characterised by an excellent degree of originality, completeness, and relevance to the sectors of both forensic and analytical sciences. For all of these reasons, it may be asserted that this doctoral thesis gives a significant contribution to the forensic-analytical research



sector, and it definitely deserves presentation as an oral dissertation for the achievement of the PhD title.

In the final conclusion I hereby state, that the PhD dissertation by Ms. Alicja Menżyk MSc, which I have reviewed, entirely fulfils the common and legal Polish requirements for the PhD degree (Polish law "O stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki", Dz. U. Nr 65, poz. 595, z dn. 14 marca 2003 r.). Based on this statement, I make an official request to the Institute of Chemistry of the University of Silesia in Katowice of its hearing to the public defence.

Yours sincerely

Prof. Paolo Oliveri, PhD