

FACULTY OF ENGINEERING OF UNIVERSITY OF PORTO

Spectral Point-of-Care for Stroke Diagnosis in Hospital Emergency Settings

Maria Leonor Martins Soares



Dissertation elaborated within the scope of the Master's in Biomedical Engineering

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October 30, 2024

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Abstract

Background: Stroke is one of the leading causes of morbidity and mortality worldwide, which makes early and accurate diagnosis essential to reduce the adverse effects of this disease. This study addresses the urgent need for rapid diagnosis of stroke, especially in hospital emergency settings. The implementation of compact, fast and easy-to-use devices, known as point-of-care diagnostic systems, could significantly reduce the diagnosis time for stroke patients.

Objectives: The main objective of this study was to evaluate and improve a Point-of-care spectral device by the quantification of blood hemogram parameters.

Methods: Spectral data from human blood samples were measured and blood cell counts were performed using the Point-of-care device. These results were then compared with cell counts obtained by standard hospital equipment (Sysmex) at the Vila Nova de Gaia and Espinho Hospital Centre, using two statistical methods: Partial Least Squares and Self Learning Artificial Intelligence. Key statistical metrics, including Pearson's correlation coefficient, standard error and mean absolute percentage error, were calculated to assess performance.

Results and Discussion: The results indicate that the Self Learning Artificial Intelligence method outperformed Partial Least Squares in all evaluation metrics, achieving the highest correlation coefficient ($R \approx 0.760$) and the lowest standard error ($SE \approx 0.329 \times 10^6$ cells/ μL) and mean absolute percentage error ($\text{MAPE} \approx 6.15\%$). The Self Learning Artificial Intelligence algorithm combined with Vis-SWNIR spectroscopy obtained a total error of around 21.7% for platelet quantification, which is within the Clinical Laboratory Improvement Amendments standards and expected error margin of analytical hematology. Although the Self Learning Artificial Intelligence method demonstrated superior performance, overall accuracy remains a challenge, highlighting the need for further optimisation.

Conclusions: This feasibility study highlights the potential of using a low-cost, compact, reagent-free Point-of-care device to accurately quantify blood cells from a small blood sample. This technique could be a potential alternative to traditional methods. However, the device was evaluated in a basic configuration that has yet to optimize the sample's absorbance and the consistency of the light path. Enhancing these factors could lead to more accurate results in the future.

Keywords: Stroke; Healthcare; Point-of-care; Vis-SWNIR spectroscopy; Blood count; SLAI.

Resumo

Contexto: O acidente vascular cerebral é uma das principais causas de morbidade e mortalidade a nível mundial, o que torna o diagnóstico precoce e exato essencial para reduzir os efeitos adversos desta doença. Este estudo aborda a necessidade urgente de um diagnóstico rápido do acidente vascular cerebral, especialmente em contexto de emergência hospitalar. A implementação de dispositivos compactos, rápidos e fáceis de utilizar, conhecidos como sistemas de diagnóstico no local de prestação de cuidados (*Point-of-care*), poderia reduzir significativamente o tempo de diagnóstico dos doentes com acidente vascular cerebral.

Objetivos: O principal objetivo deste estudo foi avaliar e melhorar um dispositivo espectral *Point-of-care* através da quantificação dos parâmetros do hemograma sanguíneo.

Métodos: Foram medidos dados espectrais de amostras de sangue humano e efetuadas contagens de células sanguíneas utilizando o dispositivo *Point-of-care*. Estes resultados foram depois comparados com as contagens de células obtidas por um equipamento hospitalar padrão (Sysmex) no Centro Hospitalar de Vila Nova de Gaia e Espinho, utilizando dois métodos estatísticos: Mínimos quadrados parciais e Inteligência artificial de auto-aprendizagem. As principais métricas estatísticas, incluindo o coeficiente de correlação de Pearson, o erro padrão e o erro percentual absoluto médio, foram calculadas para avaliar o desempenho.

Resultados e Discussão: Os resultados indicam que o método de Inteligência artificial de auto-aprendizagem superou o Mínimos quadrados parciais em todas as métricas de avaliação, alcançando o maior coeficiente de correlação ($R \approx 0,760$) e o menor erro padrão ($SE \approx 0.329 \times 10^6$ células/ μL) e erro percentual absoluto médio ($\text{MAPE} \approx 6,15\%$). O algoritmo de Inteligência artificial de auto-aprendizagem combinado com a espectroscopia do visível de ondas curtas próximo ao infravermelho obteve um erro total de cerca de 21,7% para a quantificação de plaquetas, que está dentro das normas *Clinical Laboratory Improvement Amendments* e da margem de erro esperada para a hematologia analítica. Embora o método de inteligência artificial de auto-aprendizagem tenha demonstrado um desempenho superior, a precisão global continua a ser baixa, salientando a necessidade de uma maior otimização.

Conclusão: Este estudo realça o potencial da utilização de um dispositivo *Point-of-care* de baixo custo, compacto e sem reagentes, para quantificar com precisão as células sanguíneas a partir de uma pequena amostra de sangue. Esta técnica poderá ser uma potencial alternativa aos métodos tradicionais. No entanto, o dispositivo foi avaliado numa configuração básica que ainda tem de otimizar a absorvância da amostra e a consistência do percurso da luz. A melhoria destes fatores poderá conduzir a resultados mais precisos no futuro.

Palavras-Chave: AVC; Cuidados de saúde; *Point-of-care*; Espectroscopia Vis-SWNIR; Contagem de células sanguíneas; Inteligência Artificial de auto-aprendizagem.

Acknowledgements

Firstly, I would like to express my sincere thanks to my supervisor, Rui Costa Martins, and my co-supervisor, Professor Teresa Barros, for their support, guidance and expertise throughout the development of this dissertation.

I would like to thank the professionals at the Vila Nova de Gaia/Espinho Hospital Centre, particularly the laboratory technicians who helped me at the collection centre and in the laboratory.

I am deeply grateful to Dr Tiago Gregório for facilitating communication with the hospital and all the necessary services, and for his warm hospitality and friendliness. I would also like to thank Dr Miguel Furtado for his kindness and support during my time working in the laboratory, for his help in obtaining samples and sending me the results I requested.

I am grateful to all the professors at the Faculty of Engineering of the University of Porto for their guidance and encouragement throughout my academic career.

To my family for always believing in me and to my lifelong friends for their constant support. To my engineering course friends, who have gone from colleagues to friends, thank you for being by my side and sharing the challenging moments with me.

I am truly grateful to everyone who has influenced my journey.

Finally, I would like to recognise that this work was carried out in partnership with INESC TEC - Instituto de Engenharia de Sistemas e Computadores, Tecnologia e Ciência, as part of the Master's Degree in Biomedical Engineering.

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Abbreviations

AFP	Alpha-fetoprotein
AHA	American Heart Association
AI	Artificial Intelligence
AIDS	Acquired immunodeficiency syndrome
AIS	Acute ischemic stroke
ASA	American Stroke Association
ASSURED	Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end users
Apo C	Apolipoprotein C
ANFIS	Adaptive neuro-fuzzy inference system
ANN	Artificial Neural Network
AVM	Arteriovenous malformations
Bil	Bilirubin
BLL	Beer-Lambert Law
BNP	Brain Natriuretic Peptide
CBC	Complete Blood Count
CE	European conformity
CLIA	Clinical Laboratory Improvement Amendments
COVID-19	2019 Coronavirus pandemic
CovM	Covariance mode
CT	Computed tomography
CTA	Computed tomography angiography
cTnI	Cardiac Troponin I
CTP	Computed tomography perfusion
CVD	Cardiovascular disease
CVR	Cerebrovascular reactivity
DALYs	Disability-adjusted life year
DL	Deep Learning
DSA	Digital subtraction angiography
DWI	Diffusion-weighted imaging
EC	European Community
ECGs	Electrocardiograms
ED	Emergency department
EIT	Electrical impedance tomography
EU	European Union
FDA	Food and Drug Administration
FIR	Far-Infrared Region
GDP	Gross Domestic Product

GFAP	Glial fibrillary acidic protein
HbA1C	Glycated hemoglobin
HGB	Hemoglobin
HIV	Human immunodeficiency virus
hs-cTn	Highly sensitive cardiac troponin
HTC	Hematocrit
ICPerMed	International Consortium for Personalized Medicine
INESC TEC	Instituto de Engenharia de Sistemas e Computadores, Tecnologia e Ciência
IoT	Internet of Things
IR	Infrared region
IV-tPA	Intravenous tissue plasminogen activator
IoT	Internet of Things
K3-EDTA	Tripotassium Ethylene Diamine Tetraacetic Acid
KM	Konica Minolta Inc.
LED	Light-emitting diode
LEU	Leukocytes
LFIA	Lateral flow immunoassays
LIFS	Laser-induced fluorescence spectroscopy
LocPLS	Local partial least squares
LV	Latent Variables
LVOs	Large vessel occlusions
MAPE	Mean absolute percentage error
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MIR	Middle-Infrared Region
ML	Machine learning
MMP-9	Matrix metalloproteinase 9
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
MRP	Magnetic resonance perfusion imaging
MSU	Mobile Stroke Unit
NIR	Near-Infrared Region
NIRS	Near-infrared spectroscopy
NMDA	N-methyl-D-aspartate
NIHSS	National Institute of Health Stroke Scale
NCCT	Non-contrast computed tomography
PCA	Principal Component Analysis
PCR	Principal component regression
PLS	Partial Least Squares
PLSR	Partial Least-Squares Regression
PLT	Platelets
PM	Personalized Medicine
POC	Point-of-care
POCHT	Point-of-Care Health Technologies
POCT	Point-of-care testing
PPPM	Predictive, preventive, and personalized medicine

PT	Prothrombin time
R	Pearson correlation coefficient
RI	Reference interval
RBCs	Red blood cels
REASSURED	Real-time connectivity, Ease of specimen collection, Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end users
S100 β	Calcium-binding protein, beta chain
SE	Standard error
SERS	Surface-enhanced Raman spectroscopy
SLAI	Self-Learning Artificial Intelligence
SPFS	Surface Plasmon Field-Enhanced Fluorescence Spectroscopy
SVM	Support vector machines
SVOs	Small vessel occlusions
TB	Tuberculosis
TAT	Turnaround time
tDCS	Transcranial direct current stimulation
TF	Tissue factor pathway
TIA	Transient ischemic attacks
TPC	Total platelet count
USA	United States of America
USB	Universal serial bus
UV	Ultraviolet
UV-Vis	Ultraviolet - Visible
VIPS	Volumetric impedance phase shift spectroscopy
VIS	Visible
Vis-NIR Spectroscopy	Visible-Near Infrared spectroscopy
Vis-SWNIR Spectroscopy	Visible-short-wave-near-infrared Spectroscopy
WBCs	White blood cells
WHO	World Health Organization
WO	Written Opinion

Chapter 1

Context

1.1 Background

Stroke is one of the main causes of morbidity and mortality worldwide. It is a disease in which the central nervous system does not receive enough blood flow, because the blood flow to the brain is abruptly interrupted, which causes cells to die. The interruption in the blood flow occurs when there is a rupture in an artery that carries blood from the heart to a specific region in the brain, or when a clot blocks the blood flow to that area. This blockage prevents the delivery of essential oxygen and nutrients to the brain tissue, leading to potentially severe consequences [1, 2, 3, 4, 5].

It is the second most common cause of death globally and ranks third in terms of death and disability combined, as measured by disability-adjusted life years (DALYs) [6].

The World Health Organization (WHO) reported that in 2017, around 17.7 million people died due to cardiovascular diseases, with 6.7 million of these deaths attributed to stroke [1]. Between 1990 and 2019, the global stroke burden increased significantly, with the majority of cases occurring in low and lower-middle-income countries [6]. In Portugal, stroke impacts approximately 15,200 people annually and remains the leading cause of death [7].

Stroke, a diverse condition, can be initially classified into two main categories: ischemic and hemorrhagic types (presented in figure 1.1). Ischemic stroke arises from disruptions in blood flow within vessels supplying the brain, typically due to clot formation and fat accumulation. On the other hand, hemorrhagic stroke results from the rupture of weakened blood vessels, leading to bleeding into the surrounding brain tissue. Hemorrhagic stroke encompasses intracerebral hemorrhage, which involves abnormal bleeding within the brain tissue, and subarachnoid hemorrhage, which involves bleeding in the space between the brain and its surrounding membrane (the subarachnoid space). Common causes of hemorrhagic stroke include aneurysms and arteriovenous malformations (AVM), with high blood pressure being a frequent contributor. Distinguishing between these types is crucial for effective stroke management and treatment decisions. However,

this distinction often relies on imaging technologies, which may be limited by availability, high costs, and the need for skilled personnel [4, 8].

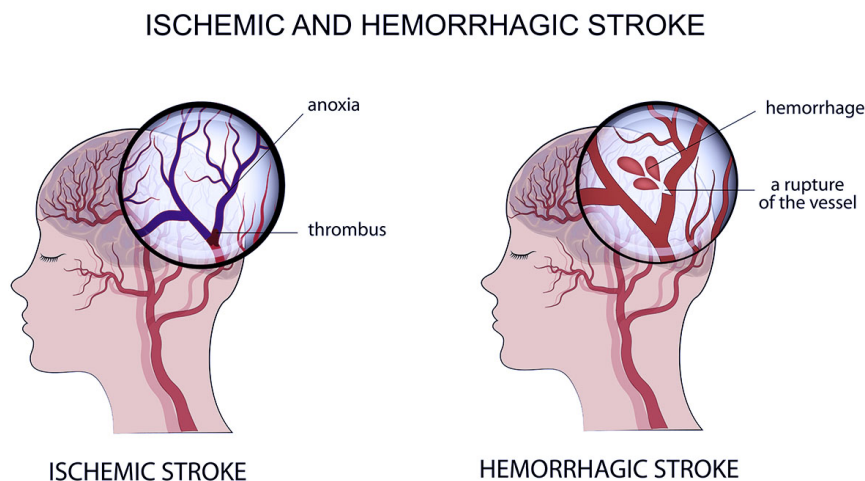


Figure 1.1: Main types of Stroke [9].

Both ischemic and hemorrhagic strokes can result in lasting disabilities like spasticity, depression, recurrent falls, and heightened vulnerability to infections. While strokes often impact the elderly, the risk extends to young individuals as well. Stroke risk is determined by a mix of factors, being more than ninety percent of strokes worldwide attributable to behavioral and metabolic factors, including a history of previous strokes or transient ischemic attacks, high blood pressure, dyslipidemia, lack of physical activity, old age, diabetes, heart disease, obesity and smoking, which pose a significant threat. The rehabilitation of stroke patients is a time-consuming process, spanning months or even years, contributing to the escalating healthcare costs in developed nations globally [4, 8].

According to the American Stroke Association, the primary symptoms of a stroke include: sudden weakness or numbness in the arm, leg, or face, particularly on one side of the body; difficulty speaking or understanding language, leading to sudden confusion; sudden visual disturbances in one or both eyes; sudden dizziness, loss of balance, or coordination; and an abrupt, intense headache with no apparent cause. In the United States (USA), approximately 87 percent of strokes are ischemic, while 13 percent are hemorrhagic [1, 2, 3, 4].

Overall, ischemic stroke has a somewhat lower mortality rate than hemorrhagic stroke, with 2.7 million deaths reported in 2016, compared to 2.8 million deaths from hemorrhagic stroke in the USA that year [8]. Acute ischemic stroke (AIS) is the most common form of stroke, making the identification of its subtypes (etiologies) the second most important factor in the clinical management and prognosis of stroke patients [4].

Stroke outcomes are mainly predicted based on key, non-modifiable factors like age and the initial severity of the stroke. However, it is becoming increasingly evident that post-stroke com-

plications, such as hemorrhagic transformations or infections, can significantly worsen these outcomes. According to the USA National Stroke Association, about 10% of stroke survivors fully recover, 25% recover with minor disabilities, and 40% are left with moderate to severe impairments requiring specialized care. Additionally, 10% of stroke survivors need ongoing care in a nursing home or similar facility, while 15% die shortly after the stroke [4].

This information underscores the significance of the condition and the need for prioritizing preventive and personalized healthcare. Throughout the twentieth century, advancements in technology transformed medicine and healthcare delivery, with instrumentation, computers, and communication technologies playing pivotal roles. This trend has continued into the twenty-first century, where innovative technologies have reshaped medical practices and the patient-provider dynamic. Globalization and technological innovations have united the world into a single interconnected community, blurring the lines between developing and developed economies. Despite the increase in global life expectancy, new challenges have emerged, particularly concerning the enhancement of quality healthcare while keeping costs manageable. In developed nations, the soaring healthcare expenditure as a percentage of gross domestic product (GDP) presents a significant challenge, while developing countries struggle to provide even basic healthcare to their vast rural populations. Addressing these challenges requires innovative solutions that prioritize affordability and accessibility for all. While the healthcare challenges vary between developing and developed nations, they both aim to extend access to health monitoring and assessment technologies. This goal is particularly crucial for regions lacking adequate healthcare infrastructure or facing geographical barriers to accessing medical facilities [10].

Preventive medicine focuses on actions aimed at preventing disease occurrence or delaying its progression, which includes promoting vaccination to combat infectious diseases, for example. Prioritizing preventive care globally, especially in low and middle-income countries, can significantly reduce healthcare costs and improve outcomes. Evidence suggests that a large percentage of cardiometabolic risks are linked to modifiable behaviors, emphasizing the importance of lifestyle changes in prevention [11].

Integrated healthcare approaches can effectively reduce disease burdens in resource-poor countries. To promote preventive medicine, innovative strategies like telemedicine are crucial: It offers opportunities for early detection, treatment access and lifestyle adherence. However, challenges such as limited access to technology and training, especially in rural areas, hinder its widespread adoption. Addressing these challenges requires collaboration among stakeholders to ensure the delivery of quality and affordable healthcare services. Community engagement and training initiatives can enhance understanding and usage of telemedicine technologies, ultimately advancing preventive medicine efforts globally [11].

By 2030, the International Consortium for Personalized Medicine (ICPerMed) envisions a healthcare landscape where individual needs take center stage, propelled by the principles of personalized medicine (PM) [12]. PM seeks to enhance healthcare by:

- More precise diagnostics and treatments: Tailored to a person's unique genetic, molecular, and environmental factors.
- Reduced healthcare costs: By focusing on prevention and avoiding unnecessary interventions.
- Increased patient empowerment: Individuals will have better understanding and control over their own health [12].

Investing in effective preventive healthcare can lead to significant cost savings by reducing the occurrence of severe illnesses and complications, as well as long-term care spending for disabilities. Early health interventions offer the greatest potential for long-term health benefits for the population. During economic downturns, governments often cut spending on preventive healthcare despite its long-term benefits, prioritizing immediate treatment for sick individuals. However, there's ongoing debate about whether the optimal level of preventive health services has been reached. Healthcare expenditure in developed countries has been increasing faster than GDP, highlighting the need for greater efficiency in resource allocation. It's important to consider a broader range of health outcomes beyond just mortality or life expectancy [13].

Allocating healthcare resources appropriately can lead to improvements in the general health of the population and strengthen human capital, leading to increased productivity and reduced future healthcare costs. Effective health interventions may also contribute to faster economic growth and higher welfare status. As aging populations present long-term care challenges, it's crucial to assess whether preventive health services are effectively impacting both health and economic performance. Investing in preventive healthcare aligns positively with socioeconomic goals, and it's beneficial to increase such investments until reaching optimal levels for economic development and social welfare. Evidence from economies with universal health coverage, like Taiwan, and studies from the USA indicate significant cost savings from community-based public health efforts, suggesting potential applicability to other countries with similar healthcare systems [13].

Balancing standardization and individualization in healthcare has always been a challenge. While personalized care is crucial, treatment protocols often rely on standardized guidelines drawn from large-scale trials. Ideally, both approaches should complement each other to tailor treatment to each patient's needs effectively. However, there's still a gap between disease-focused care and holistic healthcare, impacting ethical considerations and the economic landscape. Investing in established concepts like predictive, preventive, and personalized medicine might offer better outcomes. Shifting towards this approach requires collaboration across professions. Predictive, preventive, and personalized medicine (PPPM) offers a comprehensive framework for addressing ethical challenges in biomedicine. It's crucial for evolving healthcare systems to meet economic strains and the needs of ageing populations. PPPM represents a sophisticated approach that aims to optimize healthcare strategies for individuals, populations, and healthcare systems alike. This shift requires advancements in education, economics, and application models, for both disease treatment and health maintenance [14].

There exists a substantial body of research on cardiovascular risk factors and preventive measures at both population and individual levels. However, there are chronic disease stages that remain insufficiently addressed because they do not align with the principles of PPPM. The promotion of PPPM in managing cardiovascular disease (CVD) is a global health imperative, particularly as the burden of CVD is most severe in developed nations and rapidly increasing in many developing countries. Therefore, it is crucial to foster global collaboration in exchanging scientific insights, knowledge, and skills for predicting cardiovascular risk, implementing preventive measures, and developing personalized treatment strategies [15].

Despite being largely preventable, the global incidence of stroke is on the rise, primarily due to uncontrolled major risk factors. These risk factors, shared by other chronic diseases like heart disease and cancer, often go unaddressed, with prevention efforts being neglected and unhealthy environments prevailing [16].

Management of modifiable risk factors, such as high blood pressure and smoking, is crucial for stroke prevention, as highlighted by numerous clinical trials. While some risk factors for stroke cannot be modified, many can be addressed through effective interventions, potentially reducing the risk of future strokes. Recommendations from organizations like the American Heart Association (AHA) or American Stroke Association (ASA), provide guidance for healthcare providers in selecting evidence-based approaches for stroke prevention [17].

The figure 1.2 shows targets that can be changed to increase efficiency in the pre-intervention workflow for acute ischaemic stroke.

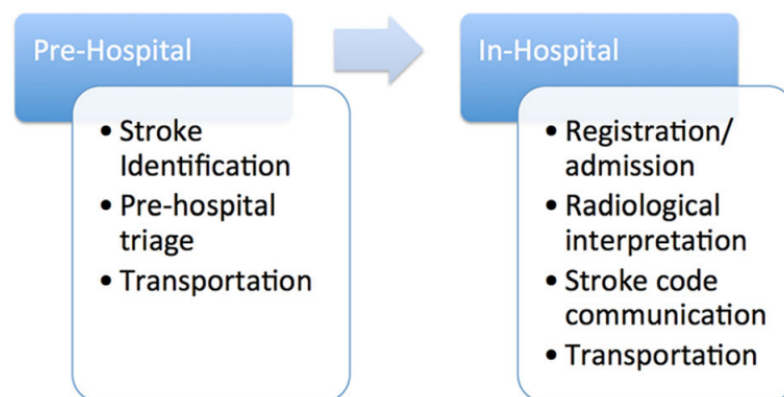


Figure 1.2: Potentially modifiable targets to enhance efficiency in the pre-intervention workflow for acute ischemic stroke [18].

Stroke is one of the most common and serious conditions encountered in emergency departments (EDs). Rapid evaluation and management of stroke patients are crucial to minimizing delays in treatment, thereby reducing morbidity and mortality. Early recognition and precise diagnosis are essential to mitigate the adverse effects of this condition [1, 2, 19]. Because brain cells die rapidly after a stroke, prompt restoration of blood flow is vital to prevent irreversible brain damage, underscoring the importance of early intervention in any effective treatment strategy. The link between the time elapsed since stroke onset and irreversible tissue damage is strongly supported

by substantial evidence. Stroke has become a treatable condition, with intravenous tissue plasminogen activator (IV-tPA) treatment improving outcomes for patients with acute ischemic stroke (AIS). The success of most stroke treatments is highly time-sensitive, yet only a small proportion of eligible ischemic stroke patients receive recanalization therapies. Delays at various stages of stroke care are associated with poorer outcomes.

The current approach to evaluating stroke patients typically involves a comprehensive clinical assessment, supplemented by neuroimaging techniques. Common practice includes performing a neurological examination using scoring systems such as the National Institute of Health Stroke Scale (NIHSS). Imaging techniques like computed tomography (CT) or magnetic resonance imaging (MRI) scans are also frequently used to provide cross-sectional images of the brain, helping to detect hemorrhage and differentiate hemorrhagic stroke. Additionally, a variety of medical tests, including blood tests, electrocardiograms, carotid ultrasounds, carotid angiography, and echocardiography, may be employed. However, there remains a need for additional diagnostic tools to improve the classification of stroke patients and better identify their medical status [1, 4].

Advancements in machine learning algorithms show promise in predicting stroke outcomes by integrating various factors, from demographic to clinical characteristics. This could lead to personalized therapeutic strategies, better patient outcomes, and ultimately, a reduction in the global burden of stroke [20].

Implementing compact, swift and easy-to-use devices, known as point-of-care (POC) diagnostic systems, could significantly reduce expenses and simplify testing procedures for stroke patients. POC testing involves conducting tests outside the traditional hospital laboratory, typically in close proximity to the patient, where the results obtained directly impact patient care and management decisions. These systems, preferably self-operating and automated, ideally administered during initial interactions with paramedics or physicians, would function on-site, requiring only a minimal blood sample. By diminishing the need for sample transportation and analysis in centralized laboratories, these devices can mitigate the likelihood of misplaced samples and logistical bottlenecks. Furthermore, they hold promise in minimizing reagent usage and expediting analysis duration. The implementation of a POC system presents a faster and more efficient approach to caring for stroke patients during their critical moments. POC offers immediate and pertinent information on-site, eliminating the need for sample processing, lengthy central laboratory testing, and results analysis procedures. Functioning as a mobile laboratory device, a POC platform is positioned directly at the patient's treatment site, allowing the same medical personnel treating the patient to conduct necessary tests. The possibilities for POC application in stroke care are substantial [4, 21, 22]. The figure 1.3 summarises the advantages of POC testing (POCT) comparing to conventional technologies. The figure 1.4 shows a comparison between conventional management of stroke patient prognosis and management with POCT tools.

While developed countries see POC technologies as a way to cut healthcare expenses and enhance effectiveness, they play a vital role in addressing diagnostic and monitoring needs in densely populated regions or rural areas, particularly in developing nations. These countries, housing the

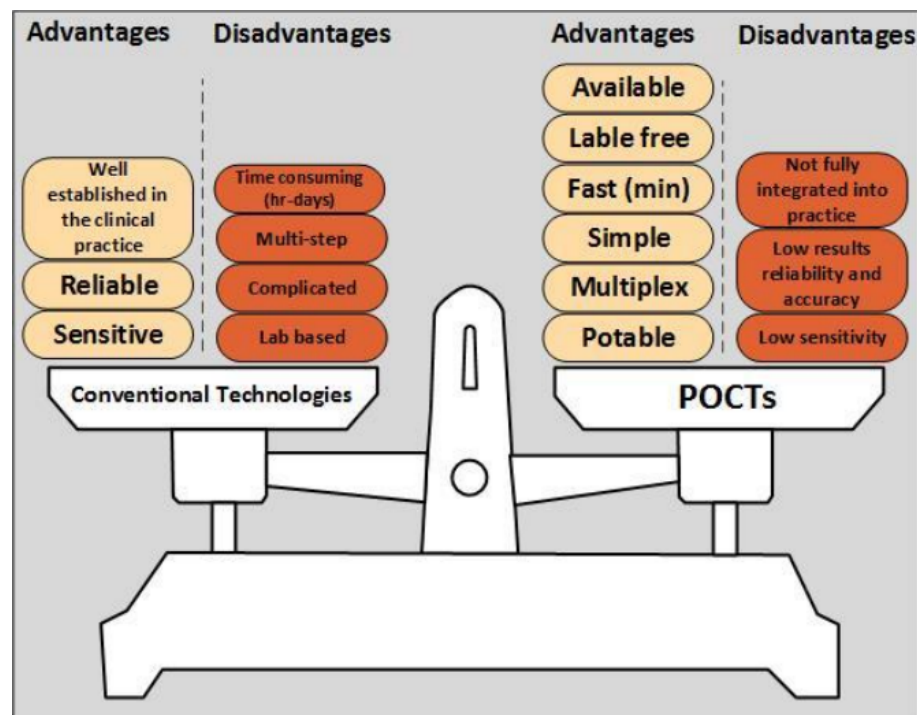


Figure 1.3: POCT vs. Conventional Technologies [4].

majority of the global population, face the fundamental challenge of delivering basic healthcare to all, especially in areas with geographical or economic limitations. Additionally, they must monitor and combat infectious diseases like Human immunodeficiency virus (HIV)/Acquired immunodeficiency syndrome (AIDS), tuberculosis and malaria, which pose significant threats, especially during potential epidemics. With aging populations in both developed and developing regions, there's a pressing need for collaborative efforts to explore POC technologies for health monitoring, assessment and treatment. These technologies hold the potential to substantially impact global healthcare and promote the well-being of society [10].

POC Health Technologies (POCHT) represent a significant shift in global healthcare, but its success relies on integration into existing healthcare models, supported by robust business cases and demonstrated improvements in patient outcomes. To ensure sustainability and economic viability, development, deployment and compliance issues must be carefully addressed. POCHT can have a profound impact on preventive, therapeutic and surveillance aspects of healthcare. While the preventive benefits may take time to materialize, such as reducing myocardial infarction rates through diabetes and cholesterol control, the therapeutic benefits can be more immediately evident, as seen in early diagnosis of conditions like myocardial infarction or eclampsia, leading to reduced mortality rates [10].

To maximize the impact of POCHT, addressing patient privacy concerns and ensuring robust infrastructure support is paramount. This includes safeguarding data integrity and security, as well as educating patients on the responsible use of these technologies for health monitoring. The diagram in figure 1.5 shows that the use of accurate, reliable and secure data can improve

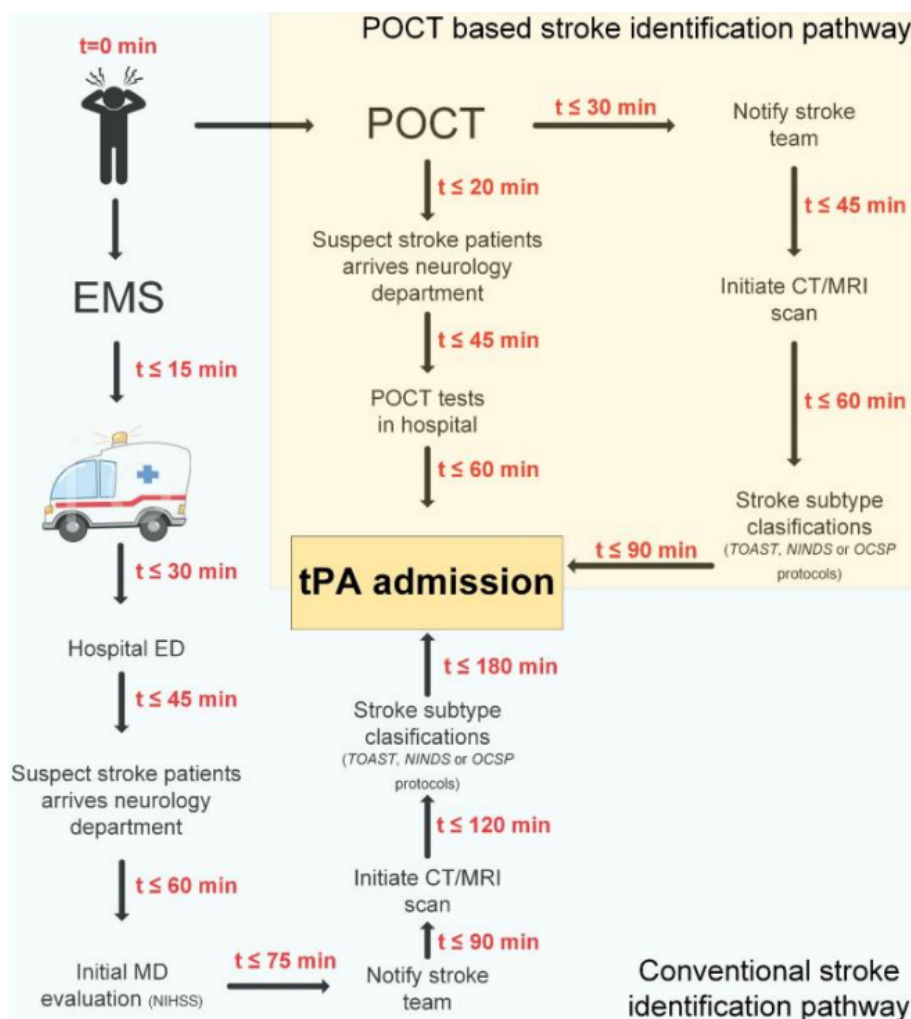


Figure 1.4: Conventional stroke patient management vs. Improvement with POC testing [4].

clinical management, treatment and therapeutic intervention and help provide quality healthcare. Adapting to these technological advancements requires patients, families and communities to embrace new approaches to healthcare delivery and recognize the advantages, such as cost savings and personalized care. Reshaping patient roles and responsibilities through patient-centered design and education is crucial. Promoting local solutions and educating patients on the benefits of POCHT, such as reduced hospitalizations and enhanced preventive care, can drive significant progress. Collaboration between developed and developing markets becomes essential to develop more accessible and effective healthcare solutions worldwide [10].

To address global healthcare challenges effectively, collaboration among stakeholders is indispensable as shown in the diagram in figure 1.6. Leveraging advanced technology and communication protocols, seamless connectivity between patients and clinicians across diverse healthcare settings can be achieved. A collaborative platform involving researchers, clinicians and industry can harness patient-centered databases to develop and deploy POC technologies, offering streamlined and affordable laboratory tests, even in resource-constrained areas. Despite the transformative potential of POC technology, hurdles remain regarding reliability, liability, and acceptance. En-

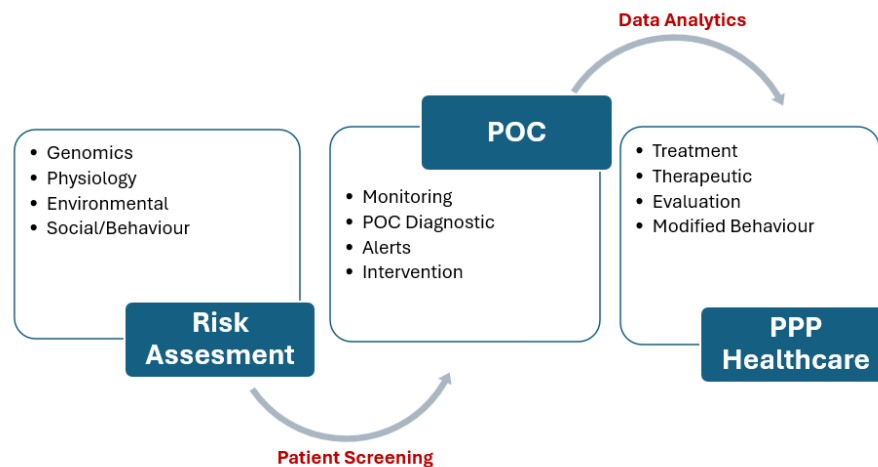


Figure 1.5: Application and impact of POC Technologies in Personalized, Preventive and Precision Medicine [10].

gaging multiple stakeholders in the early stages of research and development is crucial to address these challenges. Through collaborative efforts, the future of POC technology can be shaped, establishing a global paradigm that meets critical healthcare needs while ensuring affordability and quality care [23].

Hence, the integration of a POC device in the diagnosis of strokes can enhance medical intervention by offering valuable information. Ideally, its incorporation into emergency room stroke diagnostic protocols eliminates the need for transporting samples to a laboratory, making it a crucial and beneficial tool in emergency medicine, particularly in scenarios like emergency stroke response. The adoption of POC technology stands to enhance medical procedures, thereby contributing to an improvement in the quality of life for patients.

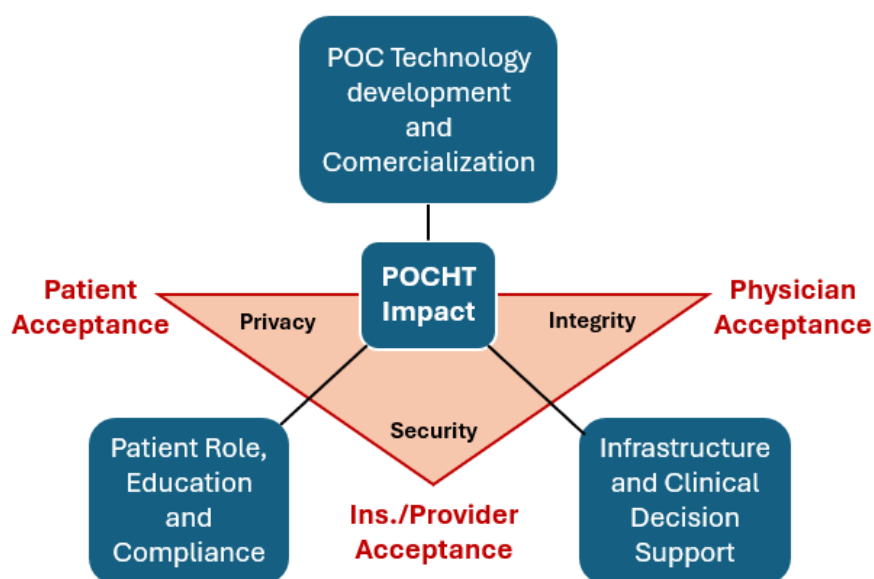


Figure 1.6: POCHT impact and paradigm with major infrastructure support and issues [23].

Laser and light-emitting diode (LED) light are now commonly used tools in medical examinations and treatments. This has led to a significant interest in understanding how light scatters when it passes through tissues. Light scattering affects various medical applications, such as determining the correct dosage for laser therapies, using light-scattering spectroscopy for diagnosing tissues, and analyzing blood for diseases. The optical properties of blood are particularly crucial for these applications. As a result, there is ongoing effort to enhance experiments, theoretical models, and numerical simulations related to blood optics [24].

Spectroscopy is a broad field that examines how matter interacts with different types of radiation to identify or characterize substances by their spectral signatures. This technique works because atoms and molecules have unique energy states that can be excited by radiation or other energy forms, resulting in distinct absorption and emission spectra for each chemical species. The main goal of spectroscopy is to understand the properties of matter and it is widely used across various fields, including the discovery of chemical elements. When samples are stimulated using energy forms like heat, electricity, light, particles, or chemical reactions, atoms in their lowest energy state absorb or emit photons of electromagnetic radiation, transitioning between specific energy levels. The energy of these photons matches the difference in energy levels. Information about the analyte is obtained by measuring either the emitted radiation as atoms return to their ground state or the absorbed/scattered radiation during excitation. Emission spectroscopy uses heat or electrical energy, while chemiluminescence spectroscopy uses chemical reactions for excitation. Both methods provide data on the analyte's identity and concentration by measuring the emitted radiant energy, typically presented graphically as a spectrum based on frequency or wavelength. When stimulated by external radiation, the sample's response—reflection, scattering, or absorption—is measured to gather further information [25].

When light travels through a medium, its intensity diminishes due to absorption. Absorbance is defined as the proportion of light intensity that is absorbed compared to the original incident light intensity. This absorption occurs because some of the light energy is transformed into heat or causes molecular vibrations within the absorbing material. A medium's ability to absorb electromagnetic radiation is influenced by several factors, including the electronic configuration of its atoms and molecules, the wavelength of the radiation, the thickness of the medium, and internal conditions such as temperature and the concentration of absorbing substances. The Beer-Lambert Law (BLL) describes how absorption is affected by the thickness of the medium or the concentration of absorbing agents [26]. When some of the incoming radiation is absorbed, it excites specific analyte species to a higher energy state. In absorption spectroscopy, the amount of light absorbed is measured across different wavelengths, providing both qualitative and quantitative information about the sample. Photoluminescence spectroscopy, on the other hand, measures the emission of photons that occurs after the absorption of light [25].

Light absorption is a vital property of cells and tissues used in diagnostics and therapy:

- **Diagnostics:** Molecules have unique spectral "fingerprints" that help identify chemical components, useful in tumor detection and assessments like pulse oximetry [27].

- Therapy: Light absorption enables lasers to produce effects for treatments such as Lasik eye surgery, laser hair removal, tattoo removal, and photodynamic therapy [27].

Effective light penetration and interaction are crucial for diagnostics, while energy deposition via absorption is key for therapy. Identifying tissue optical properties is essential for designing devices, interpreting diagnostics, and planning treatments. These properties are then used in models to predict light distribution and energy deposition [28].

When charged particles that are elastically bound are exposed to electromagnetic waves, the electric field induces their movement. Scattering takes place when the frequencies of the light do not correspond to the natural frequencies of the particles involved [26]. Light scattering can reveal both structural and functional aspects of the tissue [29].

Scattered radiation can involve either elastic or inelastic interactions with the sample. In the case of elastic scattering, the scattered radiation retains the same wavelength as the source radiation. Methods such as nephelometry, turbidimetry, and particle size analysis are based on measuring the intensity of elastically scattered light [25].

Elastic scattering is generally categorized into three main types based on the relative size of the scatterers to the wavelength: Rayleigh scattering, where scatterers are much smaller than the wavelength; Mie scattering, where the scatterers are similar in size to the wavelength; and Geometric scattering, where the scatterers are much larger than the wavelength [27].

Inelastic scattering occurs when light interacts with structural variations within a material on a scale comparable to the wavelength, leading to a scattered photon with a frequency that is either higher or lower than that of the incident photon [25, 27].

The image 1.7 shows the differences between absorption, scattering and transmission.

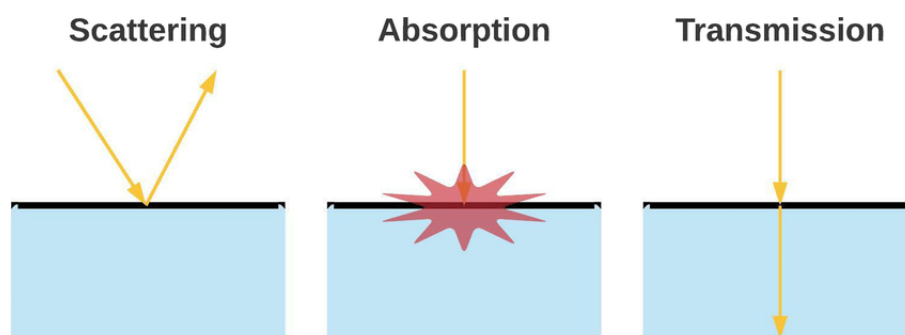


Figure 1.7: Differences between scattering, absorption and transmission [30].

Ultraviolet-Visible (UV-Vis) spectroscopy is an economical, simple, versatile, and non-destructive analytical technique well-suited for analyzing a wide range of organic compounds and some inorganic substances. This method involves measuring how light is absorbed or transmitted by a substance, which varies with wavelength. The technique is based on electronic transitions within organic molecules, where the absorbed light promotes electrons to higher energy levels. In the UV and visible regions, single atoms create sharp spectral lines due to electronic transitions, while

molecules produce broader, continuous bands because of additional excitations of rotational and vibrational energy states [31].

UV-Vis spectroscopy is particularly useful because it provides a direct, linear relationship between absorbance and the concentration of an analyte, as described by the Beer-Lambert Law. As light travels through a sample, its constituents absorb or scatter it, generating detailed spectral data about molecular structures and composition. This characteristic makes UV-Vis spectroscopy an essential tool in analytical chemistry, especially for quantifying blood cell counts, since different blood components exhibit unique spectral patterns [31].

The four main components of spectroscopy are [31]:

1. Light source to irradiate the sample.
2. Light transfer system to carry light from the source to the detector.
3. A detector that captures and transforms light into spectral data.
4. A computer that analyzes and presents the data.

The Infrared region (IR) ranges from 800 nm to 1 mm in the spectrum. Due to its extensive size, it is impractical to treat the entire region uniformly in spectroscopy. Therefore, it is categorized into three regions: near-IR (NIR), mid-IR (MIR), and far-IR (FIR) [32]. Quantitative infrared (IR) absorption techniques differ from ultraviolet-visible molecular spectroscopy due to the increased complexity of IR spectra, narrower absorption bands, and the limitations of IR instruments. Near-infrared (NIR) spectroscopy is distinguished by the presence of weak or very weak absorption bands, setting it apart from other spectral regions. This distinction is particularly valuable because the NIR region serves as the only high-transmission window for radiation [25]. Unlike mid-IR spectroscopy, NIR absorption spectra are less suitable for identifying compounds but are particularly effective for quantitatively analyzing compounds with functional groups containing hydrogen bonded to carbon, nitrogen, or oxygen. These compounds can often be quantified with an accuracy and precision comparable to ultraviolet-visible spectroscopy, rather than mid-IR spectroscopy [25].

Spectroscopy is a widely utilized technique in various fields, including medical physics. Similarly, artificial intelligence (AI) is increasingly integrated into modern technologies.

The work carried out in this thesis demonstrated the testing and improvement of a prototype spectral device POC for quantifying blood cells in human blood, using signal processing and advanced artificial intelligence systems dedicated to multi-scale spectral information processing.

To address the complex nature of blood spectral data, a novel artificial intelligence technique known as Self-Learning Artificial Intelligence (SLAI) was developed. This method is based on a Covariance Mode (CovM) search, which identifies clusters of samples in the feature space that exhibit similar interference patterns, particularly those sharing the same covariance eigenvector or CovM. These modes capture stable, correlated variations between specific spectral characteristics

and compositional variables. Utilizing this information enables precise quantitative analysis of unknown samples by determining which CovM or combination of modes aligns most closely with the sample's position within the feature space. This stable covariance is intrinsically linked to the Beer-Lambert Law (BLL), facilitating the identification of complex, multi-scale interferences among blood components for accurate blood cell quantification [33].

Spectroscopy allows for the use of small, cost-effective, low-energy components with a simple acquisition process. This makes it feasible to incorporate computing power for integrating AI into the device and developing an Internet of Things (IoT) platform to enhance portability and user-friendliness. Its reagent-free operation and minimal blood volume requirement further reduce costs and simplify sample preparation. This technology is well-suited for home use, enabling regular monitoring of chronic or bedridden patients, and can be easily transported to regions with limited medical resources for population health assessments. It offers significant benefits to primary care, nursing, and epidemic control efforts. Additionally, the concept can be extended to wearable technologies, such as smartwatches, or smartphones equipped with attachable gadgets to collect and analyze spectral data, bringing healthcare even closer to users. The spectroscopy technique employed was Visible-short-wave-near-infrared (Vis-SWNIR) spectroscopy, which provides both physical and chemical information. This method shows great potential for POC applications, as it does not require reagents or extensive sample preparation [33].

POC spectroscopy works within the spectral information domain, allowing for the analysis of specific characteristics of an analyte at the Covariance Mode level when similarities in the data are established. When local information is adequately represented, unique details can be extracted, thereby improving the analytical specificity of POC spectroscopy for complex biological samples [34].

1.1.1 Blood cells

Blood is a specialized bodily fluid composed of four main components: plasma, red blood cells, white blood cells, and platelets. Plasma, the liquid part of the blood, contains water, sugars, fats, proteins, and salts. Its primary role is to transport blood cells throughout the body, along with essential nutrients, waste products, antibodies, clotting factors, hormones, and proteins that regulate fluid balance. Red blood cells (RBCs) are the most abundant type of blood cell, making up around 40 to 45% of the blood's volume. They have a unique biconcave, disc-like shape with a flattened center and are filled with hemoglobin, a protein that facilitates the transport of oxygen from the lungs to the body's tissues and returns carbon dioxide to the lungs for exhalation. White blood cells (WBCs), although much less numerous—comprising about 1% of the blood—are vital for the body's defense against infections. Neutrophils, the most prevalent type of WBC, are crucial in mounting an immune response. Together, these elements make blood essential for transporting oxygen, nutrients, and waste products, as well as for defending the body against disease [35].

A complete blood count (CBC) test, or hemogram, provides key information about the different types and quantities of cells in the blood, including red blood cells, their volume percentage (hematocrit), protein content (hemoglobin), as well as white blood cells and platelets. The results

of a CBC can help diagnose various conditions, such as anemia, infections, and other disorders [35].

Platelets, the smallest cells in the blood, play a key role in clotting and repairing blood vessels. An elevated platelet count, known as thrombocytosis, can result from abnormal cell production in the bone marrow or ongoing health conditions like anemia or inflammation. Diagnosing thrombocytosis relies heavily on the total platelet count (TPC). This condition raises the risk of blood clots forming in blood vessels, which can lead to serious, potentially life-threatening events such as strokes. TPC is usually measured in a laboratory using flow cytometry techniques, such as laser scattering or impedance detection. Due to the limitations of automated hematology in accurately determining TPC, clinical pathologists often use manual counting with microscopy as a standard quality assurance method [33].

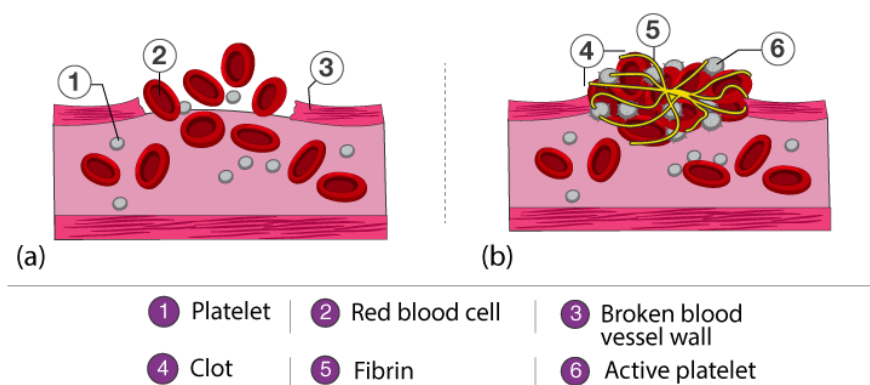


Figure 1.8: Platelets and Coagulation [36]

Blood cells counting has already been carried out using a spectral POC device in animals, but its use for cellular quantification in human blood was explored in this work, through the analysis of light spectrum [33].

1.2 Motivation

Currently, stroke is diagnosed essentially by imaging methods. As mentioned earlier, rapid diagnosis of this disease is crucial for reducing mortality and morbidity. For this reason, it is important to find more effective and rapid diagnostic methods for strokes in hospital emergency settings. This requires innovative approaches.

POC tools, currently used in many different contexts, allow rapid feedback on the results of the tests they perform and it may be important to have this type of tool to diagnose time-sensitive diseases such as stroke.

Previous studies have shown that it is possible to quantify different blood cells, such as leukocytes, platelets and red blood cells by spectroscopy, in cats and dogs, using a Spectral POC device [33, 37, 38]. This is done through information obtained from the spectrum, which can be chemical, through the absorption of light, that gives information about the cellular composition of the

blood sample, and physical, such as scattering, which gives information about cell aggregation, as shown in scheme 1.9 [38].

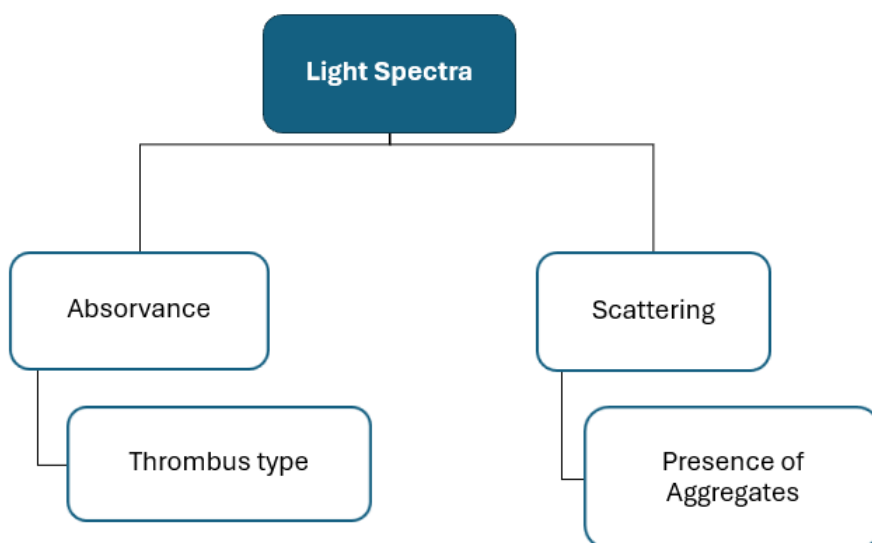


Figure 1.9: Scheme representing information obtained from the spectrum

When blood samples contain aggregates or thrombi, it results in scattering effects in the light spectrum, which can be detected. As mentioned earlier, an elevated platelet count in the blood can contribute to the formation of aggregates, increasing the susceptibility to diseases like stroke. However, it's important to note that the presence of these aggregates doesn't necessarily guarantee the development of a stroke. Thrombi, on the other hand, also impact light spectrum scattering, and larger thrombi in the process of impeding blood flow to the brain can contribute to strokes. Therefore, investigating this process is crucial for understanding its relevance.

In these studies, an artificial intelligence method called Self-Learning Artificial Intelligence was used. This method is based on the ability to distinguish the information of the different blood components obtained through spectroscopy, by reducing the interference that may be caused by different blood components that absorb in the same range of the spectrum to be observed, leading to an one-dimensional relationship between the compound to be quantified and its spectrum without interferences [33, 37, 38]. A POC device was created to directly measure white blood cell counts in canine blood using visible-near infrared spectroscopy, tailored specifically for veterinary use. This device compares traditional chemometric methods with self-learning artificial intelligence. The spectral data collected contained information on white blood cell counts, allowing for effective differentiation and showing a strong correlation between the hemogram data and the principal component scores derived from the spectra [38].

Furthermore, the device has also been utilized to perform total platelet counts in the blood of dogs and cats through advanced spectroscopic analysis, employing a self-learning artificial intelligence processing method. The findings indicate that spectroscopy holds promise as a diagnostic approach for detecting elevated platelet levels directly in whole blood samples [33].

The successful application of this study in animals suggests the potential for its implementation

in humans to measure blood cells, namely platelet quantities, and identify platelet aggregates and/or thrombi.

1.3 Objectives

The general aim of this work is to integrate a POC device into a medical procedure, furnishing doctors with pertinent information.

The primary objective was to demonstrate the feasibility of performing blood cell counts in humans, building on previous findings that established this correlation in animals [33].

Advanced artificial intelligence systems, SLAI, together with signal processing dedicated to multi-scale spectral information, are employed to deal with spectral interference and obtain meaningful information.

The purpose is for the sensitivity and specificity of the device to be as high as possible in order to achieve reliable results and really add value to the prognosis of this disease.

In the course of this research, the specific objectives are:

- Optimization of blood cell count data collection results by hospital equipment;
- Spectrum measurement of blood samples and quantify blood cells using POC device;
- Build a database with the information collected;
- Comparative analysis of the results obtained;
- Demonstrate the feasibility of blood cell counts in humans.

1.4 Outline

As previously stated, the main goal of this work is to incorporate a POC device seamlessly into a medical procedure. To this end, the work was divided into several stages which are represented in 5 chapters:

- Chapter 1 introduces the background theory supporting this work, the motivation, relevance and the objectives of the study, as well as outlines the organization of the thesis.

- Chapter 2 provides a comprehensive review of the current advancements in stroke assessment techniques, spectroscopy techniques in the medical and cardiovascular domain, the progress of POC Technology in medical and cardiovascular applications, and the role of Artificial Intelligence in the medical and cardiovascular field so that the novelty of the critical research developments presented in this project can be understood.

- In Chapter 3, the materials and methods that guide the achievement of the objectives are presented.

- In Chapter 4, the results obtained from the statistical analysis are approached, compared and discussed.

- In Chapter 5, the main conclusions obtained from the developed work are summarized. Future work and perspectives are outlined.
- Finally, the bibliography gathers all the references used in the elaboration of this thesis.

Chapter 2

State of the Art

This chapter focuses on contextualising the current methods of stroke diagnosis and recent advances. It also covers the techniques and technologies used in this research work, such as spectroscopy, POC technology and artificial intelligence, the latest advances and their relationship with medicine and stroke.

2.1 Current state-of-the-art stroke assessment techniques

The commonly accepted approach for assessing a stroke incorporates the use of both brain and neurovascular imaging, in conjunction with a clinical evaluation of stroke severity using the NIHSS [39, 40].

Advancements in neuroimaging have progressed simultaneously, leading to enhanced evaluation of the risk-benefit profile before treatment, efficient triage to suitable therapies, and the ability to rule out conditions that mimic strokes [41].

The current standard for stroke diagnosis is non-contrast computed tomography (NCCT) of the head, which is preferred because it is quick, widely accessible, and cost-effective. NCCT can confirm a diagnosis of hemorrhagic stroke, such as intracerebral or subarachnoid hemorrhage, with over 95% accuracy. It is useful for identifying conditions that may contraindicate certain treatments, like hemorrhage or large infarcts, and for distinguishing between strokes and other conditions that present with similar symptoms, such as brain tumors. Although NCCT is effective for diagnosing major strokes, its lower spatial resolution limits its sensitivity for detecting minor strokes. Additionally, even among experienced professionals, there is only moderate agreement in the interpretation of NCCT images [39, 40, 41].

Protocols including Computed tomography (CT), Computed tomography angiography (CTA) and Computed tomography perfusion (CTP) are commonly employed in the diagnosis. Many hospitals now have access to computed tomography protocols to pinpoint the blocked blood vessel and evaluate collateral blood flow in acute ischemic stroke (AIS). Although the sequential imaging process for these protocols can be time-consuming, advancements in automated software are helping ameliorate this issue [39, 41].

Digital subtraction angiography (DSA) is an older technology that is time-consuming, involves invasive procedures, and is not as commonly utilized nowadays. In comparison to CTA, DSA is more invasive. Studies indicate that the agreement among different assessors in determining the type of occlusion using CTA is better than that achieved with DSA. As a result, CTA is more widely employed due to its less invasive nature and higher reliability in determining the type of blood vessel blockage [39].

Magnetic resonance imaging (MRI) is more sensitive than other imaging methods for detecting acute ischemic stroke. However, its use is often restricted due to limited availability and is typically reserved for situations where there is no immediate need for treatment, often serving as a follow-up imaging tool. Quickly assessing acute ischemic stroke (AIS) patients for MRI suitability can be difficult, particularly in those with severe neurological symptoms. Although MRI is susceptible to motion artifacts, requires longer imaging times, and is not always readily available, many stroke centers still use it effectively for acute stroke triage [39, 40, 41].

Magnetic resonance imaging (MRI) can be supplemented with magnetic resonance angiography (MRA) and magnetic resonance perfusion imaging (MRP). MRA can be an alternative to computed tomography angiography (CTA) in cases where contrast agents are contraindicated or when CTA results are unclear. Although not as frequently used as CTA, MRA is effective for assessing brain perfusion and quickly detecting large vessel occlusions (LVO). Research shows that contrast-enhanced MRA is more reliable than non-contrast MRA for identifying blockages in intracranial arteries. Additionally, diffusion-weighted imaging (DWI) offers higher sensitivity and specificity for determining the volume of infarcted tissue. DWI significantly improves early infarction detection compared to CT and is currently the most sensitive method for identifying infarction at its earliest stage [39, 41, 42].

2.1.1 Advancements in stroke detection: recent progress in medical and technological approaches

Stroke clinicians frequently encounter diagnostic complexities. A crucial diagnostic test for stroke is necessary not only to accurately recognize conditions that mimic stroke but also to assist in differentiating between hemorrhagic and ischemic strokes. The ability to distinguish between these two types of strokes is significant as it informs treatment decisions. Intravenous thrombolysis, a beneficial treatment for ischemic stroke, is contraindicated in cases of hemorrhagic stroke [43].

Extensive research and development are underway to design innovative instruments that are practical, portable, and cost-effective while providing valuable support to existing diagnostic methods in intensive care units. Several new portable devices for stroke diagnosis, showing promising sensitivity and specificity, are currently being developed. These emerging diagnostic tools utilize alternative imaging techniques such as volumetric impedance phase-shift spectroscopy, microwave tomography, and Doppler ultrasound. They are at various stages of development, with prototypes undergoing testing to evaluate their effectiveness [39].

2.1.1.1 Physical methods

The Strokefinder™ MD100, developed by Medfield Diagnostics AB (Gothenburg, Sweden), is a microwave-based device designed to be mounted on a stretcher. It can detect intracranial hemorrhage with a high sensitivity of 100% and a specificity of 75%. Unlike ultrasound and impedance methods, microwave technology can easily penetrate the skull. This device uses low-power, non-ionizing microwave radiation and applies mathematical algorithms for signal processing [39].



Figure 2.1: Strokefinder MD100 is a device for decision-support to assist in clinical evaluation and triage of suspected intracranial injuries in the acute situation. [39]

The SONAS® device, created by BURL Concepts (San Diego, California), uses transcranial ultrasound in combination with intravenous microbubble contrast to detect potential large vessel occlusions (LVOs). Designed for use in the field, this technology aims to speed up the diagnostic process and help quickly direct patients to appropriate medical facilities. The SONAS® device features a headset with ultrasound transducers on each side of the head—one for transmitting and the other for receiving signals. This portable device is currently in clinical trials [18, 39] and is depicted in figure 2.2 below.

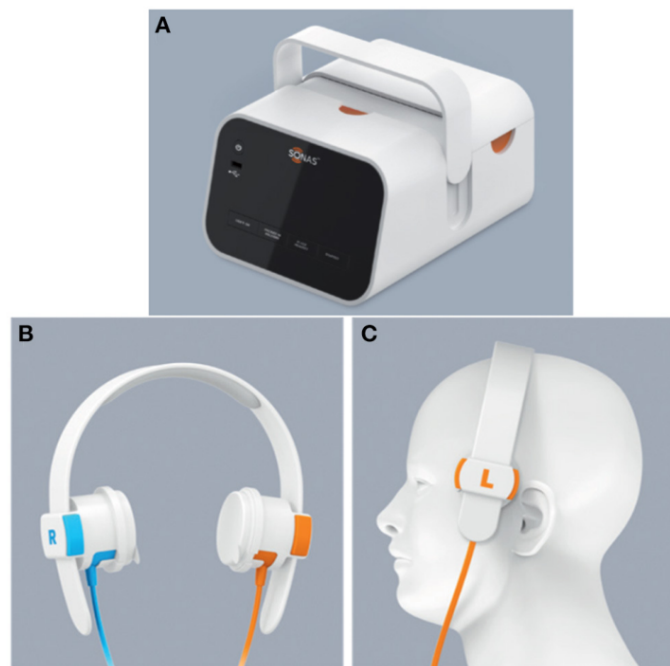


Figure 2.2: The SONAS® device (BURL Concepts, California, USA), (A) Signal generator, (B) headphones and (C) headphone placement over patient's head [39]

The Lucid™ Robotic System, developed by Neural Analytics (California, USA) utilizes transcranial Doppler ultrasound. It has demonstrated the ability to distinguish individuals with LVOs with a high sensitivity of 100% and specificity of 86% when compared to healthy controls. In the diagnosis of LVO in patients with suspected stroke, the system exhibited a sensitivity of 91% and a specificity of 85%. Regulatory approvals include Food and Drug Administration (FDA) approval in the the United States of America (USA) and European conformity (CE) marking in the European Union [39, 44].



Figure 2.3: The Lucid™ Robotic System (Lucid™ M1 Transcranial Doppler Ultrasound System® and NeuralBot™ System) is a robotically assisted ultrasound system for brain health assessment [39]

Electrical impedance tomography (EIT) is an affordable, safe, and portable imaging method relying on impedance. It produces images reflecting the internal electrical impedance of tissues by gauging radiofrequency currents flowing through electrodes placed on the tissue's surface. Scientists are investigating the application of EIT in brain imaging, especially for swift stroke diagnosis. Experiments utilizing EIT in animal models have revealed distinct impedance variations between healthy and stroke-affected brains [39, 45].

The Visor® device, developed by Cerebrotech (California, USA), employs volumetric impedance phase shift spectroscopy (VIPS). In a study with 248 participants, it demonstrated high sensitivity (93%) and specificity (92%) in differentiating severe strokes from minor strokes due to large vessel occlusions (LVOs). This non-invasive device is worn on the patient's head to detect variations in bioimpedance. By utilizing VIPS technology, it identifies bioimpedance patterns across various tissues. The device functions by emitting low-energy radio waves from the back of the head, which are then received by a sensor positioned at the front. The Visor® has received FDA approval for use in the USA and holds a CE mark for distribution within the European Union [39, 44].

Figure 2.4 provides an image of the Visor® device.



Figure 2.4: The volumetric impedance phase shift spectroscopy (VIPS) device [44].

As previously shown, brain imaging is a pivotal biomarker in the management of acute stroke. However, drawbacks such as imaging expenses, limited availability, contraindications, and the expertise needed to interpret advanced imaging results exist. The inclusion of less costly and more readily accessible stroke biomarkers identified in blood would be a valuable supplement to the tools available to stroke clinicians [46].

Timely identification of individuals experiencing acute ischemic stroke is crucial due to the time-sensitive nature of revascularization therapies. Currently, intravenous thrombolysis is limited to a window of 4.5 hours [47, 48], while endovascular thrombectomy can be considered within a 24-hour time frame [49]. Another significant challenge in stroke diagnosis involves determining the underlying cause of the stroke. Additionally, the pathophysiological mechanisms contributing

to brain damage and repair during human stroke are not fully understood, impeding the development of supplementary drug therapies to enhance the recovery process. Researchers are exploring various molecules as potential blood biomarkers for diagnosing stroke [43].

2.1.1.2 Biochemical methods

Blood is a circulating liquid that flows through the blood vessels under pressure. In the event of vascular injury, it is crucial to quickly transform the escaping blood into a gel-like substance, known as a clot, to stop further blood loss. The plasma, which is the liquid component of blood, contains various soluble proteins. These proteins work in a sequential cascade of enzyme activation, ultimately leading to the formation of a fibrin clot [50]. A schematic of the blood clotting process is shown on the figure below adapted from [50].

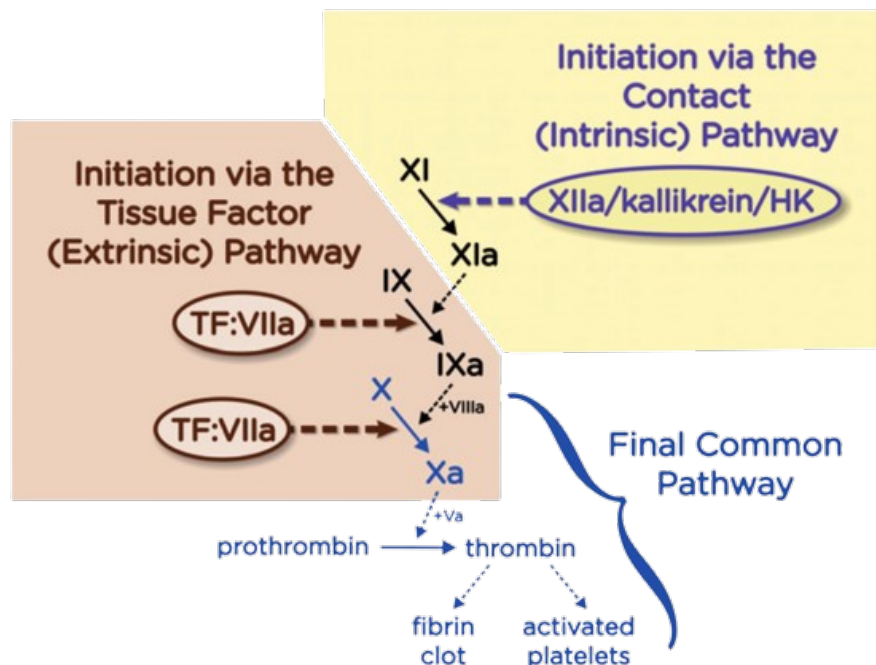


Figure 2.5: Scheme of the blood clotting process.

A stroke is primarily the result of a blood clot that obstructs the blood vessels, preventing the brain from receiving oxygen and nutrients. While blood clotting is a natural process that aids in vessel repair during bleeding, it can lead to issues like a stroke by impeding blood flow when clots form. Hence, recognizing and identifying blood clots is crucial for diagnosing strokes [51, 52].

Hemostasis is the natural process that helps seal a blood vessel injury to prevent blood loss following trauma. Thrombosis, in contrast, is an abnormal condition where the coagulation cascade is activated within a blood vessel, leading to the formation of a blood clot, known as a "thrombus." This clot can obstruct blood flow in the vessel, and in severe cases, it may completely block the flow, causing ischemia and eventual tissue death, as seen in stroke. The blood clotting cascade is initiated through two primary pathways: the extrinsic pathway, which depends on tissue factor to start clotting, and the intrinsic pathway, which can be triggered by specific surfaces without tissue

factor. Both pathways are involved in hemostasis and thrombosis, with the extrinsic pathway being essential for normal clotting, while the intrinsic pathway plays a role in thrombotic conditions [50].

Assessing blood clotting functions, including prothrombin time, activated partial thromboplastin time, and d-dimer, is a standard practice for diagnosing strokes. However, these tests can lack precision because their results may vary depending on the reagents or blood volume used. Consequently, researchers have investigated blood biomarkers for stroke diagnosis as a more cost-efficient method that can provide a rapid and accurate diagnosis [51].

Biomarkers are measurable and objective signs that serve as indicators for assessing normal or abnormal biological processes, gauging responses to treatments, and predicting potential outcomes. In clinical practice, numerous blood biomarkers are already employed to inform decision-making [43].

An ideal set of characteristics for stroke blood biomarkers should prioritize high specificity and sensitivity similar to the characteristics of biomarkers observed using imaging tools. This is crucial for effectively distinguishing strokes from conditions that mimic strokes and for discerning between hemorrhagic and ischemic strokes. The ideal biomarkers should be associated with cellular processes that are specific to ischemic strokes, a task that presents considerable challenges. Proteins typically abundant in the brain but not normally found in the blood emerge as clear and promising candidates for such biomarkers [39].

The range of potential biomarkers is steadily growing, now including proteins, RNA, lipids, and metabolites. Although these biomarkers show potential for improving stroke diagnosis and patient care, none have yet demonstrated the ideal balance of sensitivity, specificity, speed, accuracy, and cost-effectiveness needed for regular use in stroke management. This highlights the critical need for further research in this field. Enhanced standardization of clinical, laboratory, and statistical methods across different centers is crucial to maximizing the effectiveness of these biomarkers [43].

In the course of a stroke, the dysfunction of the blood-brain barrier diminishes the separation between the fluids within the brain and the peripheral system. This alteration permits the migration of brain-derived factors into the bloodstream, a movement that is typically restricted. Promising blood biomarkers for indicating stroke in individuals with symptoms of acute neurological dysfunction include glial proteins like glial fibrillary acidic protein (GFAP) and S100 calcium-binding protein B (S100 β), both of which serve as structural astrocytic proteins. Additionally, research suggests that the levels of GFAP and S100 β may have the potential to differentiate between acute ischemic stroke and brain hemorrhage [39].

GFAP stands out as the leading candidate thus far for distinguishing between hemorrhagic and ischemic strokes. Numerous studies conducted in diverse clinical settings have consistently demonstrated a delayed release of GFAP in patients experiencing ischemic stroke when compared to those with hemorrhagic stroke. This disparity in the kinetics of GFAP release between the two types of strokes creates a diagnostic timeframe, providing a distinctive window for diagnosis [39, 43, 46].

While the literature lacks a clear consensus, S100 β has exhibited promising potential as a biomarker for distinguishing AIS from brain hemorrhage. There is evidence suggesting that peripheral S100 β levels provide an estimate of overall brain damage, not limited specifically to strokes, as elevated levels are observed in various neurological conditions. Nonetheless, when coupled with the manifestation of sudden and acute neurological symptoms, S100 β could prove diagnostically useful for identifying strokes. Elevated S100 β levels in the blood of ischemic stroke patients are linked to an increased risk of hemorrhagic transformation following thrombolysis treatment, making this protein particularly interesting for guiding clinical decision-making after a stroke. However, further research is necessary to validate these findings [39].

Numerous studies and comprehensive analyses have identified additional potential biomarkers for diagnosing strokes. These include matrix metalloproteinase 9 (MMP-9), Brain Natriuretic Peptide (BNP), N-methyl-D-aspartate receptor proteins, and apolipoproteins [39].

Expectation has been elevated regarding MMP-9 as a potential biomarker for diagnosing strokes, given its involvement in responding to brain injuries through the degradation of the extracellular matrix. Acutely measured concentrations of MMP-9 have been associated with larger infarct sizes, more adverse neurological outcomes, and complications related to hemorrhagic transformation [46].

BNP is a neurohormone composed of peptides, and various clinical studies have substantiated its role in regulating hemodynamics during the acute phase of ischemic strokes [53]. Elevated levels of B-type natriuretic peptide (BNP) in the serum of stroke patients are linked to cardioembolic infarction, higher mortality rates, and signals of a heightened risk for a subsequent stroke [4].

Following a stroke, there is an increase in auto-antibodies against NMDA glutamatergic receptors, specifically targeting the NR2A/NR2B subunits. These autoantibodies, associated with neurotoxicity, demonstrate elevated levels and effectively differentiate patients with ischemic events. The sensitivity of this distinction is 97%, and the specificity is 98% [46].

Certain members of the apo-lipoprotein family have been explored as potential biomarkers for diagnosing ischemic strokes. Within the initial six hours of symptom onset, concentrations of Apo C-I and Apo C-III were observed to be higher in ischemic strokes compared to intracerebral hemorrhages. Both demonstrated the ability to effectively distinguish between ischemic strokes and intracerebral hemorrhages. Specifically, Apo C-III achieved this with a sensitivity of 94% and a specificity of 87% [46].

2.1.2 Future perspectives

Currently, stroke diagnosis and assessment, including its subtypes, largely rely on standard imaging techniques like CT and MRI. However, the availability of these imaging tools is limited in low-income areas and smaller healthcare settings, such as local hospitals and clinics. Delays in stroke evaluation and treatment are associated with poorer patient outcomes. Developing portable diagnostic devices and biomarker panels that can effectively differentiate between hemorrhagic and ischemic stroke subtypes could become valuable tools if shown to aid clinical decision-making. These devices could enable earlier stroke assessments by first responders in emergency

situations. The high cost of CT and MRI scanners, along with restricted access to imaging centers and specialists—especially in the world’s poorest regions—leaves many strokes undiagnosed and untreated. Early delivery of thrombolytic and endovascular therapies is essential for achieving better patient outcomes [39].

2.2 Current state-of-the-art point-of-care technologies

Recent technological advancements in laboratory medicine have significantly enhanced clinical diagnostics and monitoring, raising the standard of patient care. These innovations have brought substantial value to laboratory medicine in the healthcare sector. POC technology has been increasingly implemented in clinical environments because of its capability to deliver crucial laboratory test results rapidly. Although there were initial concerns in the medical community about the accuracy and precision of these devices, recent developments have resolved these issues, boosting confidence in their reliability. The clinical benefits of POC technology have been thoroughly documented and widely recognized, leading to a steady rise in its use across Europe and globally [54, 55].

Historically, central laboratories were essential because of the large and complex equipment needed for many standard medical tests. However, technological advancements have made it increasingly feasible to perform routine clinical tests outside traditional laboratory environments, closer to the patient’s point of care, while still ensuring a satisfactory level of accuracy. POC testing refers to diagnostic procedures conducted away from a central laboratory, typically at or near where the patient is located. By integrating sample collection and data analysis at the same site, POC testing minimizes delays associated with sample transport and processing, enabling healthcare providers to receive rapid test results.

POC devices offer significant advantages in terms of portability and speed, allowing healthcare providers to conduct, analyze, and make decisions based on test results within minutes, a much faster process than sending samples to a central laboratory. When used effectively, POC devices have been shown to reduce strain on healthcare systems, improve the quality of care, and enhance accessibility and affordability for patients. They also streamline workflow efficiency and provide considerable financial benefits. As these devices continue to advance, they promise a future of more efficient and effective healthcare delivery [55, 56, 57].

There is a growing demand for simple, non-intrusive methods to diagnose or screen for significant medical conditions. The capability to swiftly, non-invasively, and conclusively identify the early stages of a disease offers numerous benefits. These advantages include the prompt implementation of therapeutic interventions, resulting in reduced morbidity and mortality, and the optimization of economic resources in healthcare systems facing overwhelming demands. There is a distinct requirement for novel and cost-effective alternative analytical methods, particularly as screening tools, serving as a reliable indicator, especially in settings with limited economic resources and a need for POC diagnostic evidence [58].

POC testing encompasses several distinct stages: the pre-analytical phase, which involves selecting the appropriate testing method and collecting samples; the analytical phase, which focuses on detecting specific biological signals and converting them into quantifiable measurements; and the post-analytical phase, which includes analyzing the data, displaying the results, and storing or transmitting the information afterward [59].

Progress in POC diagnostics has been propelled by several trends, including the miniaturization of analytical chemistry and genomic techniques, the large-scale integration of fluid handling technologies similar to microelectronics, and their application to global health. A notable recent factor is the rapid growth of consumer electronics and connected devices. In today's interconnected world, the POC ecosystem has become remarkably diverse, highlighting a trend that could transform healthcare delivery globally [60].

A major obstacle to the widespread use of POC devices is the regulatory approval process, even though their effectiveness and safety have been well-established. However, as regulatory agencies become more familiar with these technologies, it is expected that approval processes will become more efficient. In 2003, the World Health Organization introduced the ASSURED criteria to guide the development of POC tests, highlighting qualities such as affordability, sensitivity, and ease of use. In 2018, these criteria were expanded to REASSURED, adding elements like real-time connectivity, simplicity in specimen collection, and environmental sustainability. The goal of these criteria is to balance accuracy, cost-effectiveness, and accessibility, ensuring that diagnostic tools can be effectively implemented across various healthcare settings [61].

Additionally, the future of POC devices will be influenced by advancements in AI and machine learning (ML), which hold the potential to enhance device accuracy and personalize results. These technologies are expected to revolutionize diagnostic tools, including predictive models and personalized health assessments. Overall, the evolving landscape of POC devices promises exciting advancements that will reshape healthcare delivery [56]. Within Europe, POC devices fall under the regulatory framework established by the 1998 European Directive 98/79/EC concerning in vitro diagnostic medical devices [55].

2.2.1 Point-of-care technologies in the medical field

POC technology encompasses a diverse range of procedures and technologies, frequently relying on either electrochemical or optical detection methods. This includes devices designed for assessing hemoglobin concentrations, conducting differential complete blood counts, performing pregnancy tests, measuring blood glucose levels, analyzing cardiac biomarkers, conducting coagulation tests, assessing platelet function, detecting group A streptococci, conducting HIV tests, screening for malaria and various other applications. Current research in the development and design of POC devices is concentrated on reducing device size while maintaining high sensitivity and accuracy in results. Successful examples of POC devices include glucose biosensor strips and lateral flow immunoassay strips, with the pregnancy test being one of the most widely recognized. [4, 55, 62].

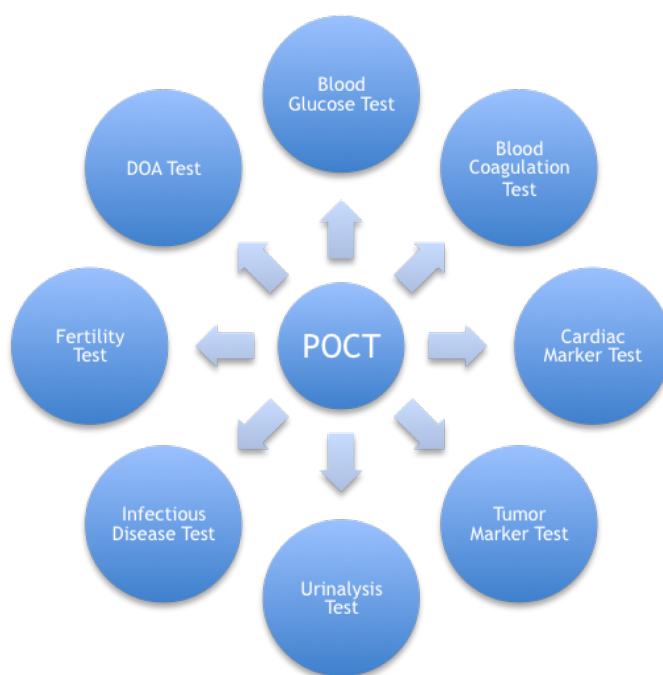


Figure 2.6: Usefulness of POC Testing [63].

POC testing is divided into three main categories: dipsticks, lateral flow immunoassays (LFIA), and microfluidics. Dipsticks, radioimmunoassays, and latex agglutination assays were precursors to lateral flow tests. LFIA relies on the principle that a sample interacts with a pre-loaded, labeled antibody on materials like polymer, nitrocellulose, or paper strips. In contrast, microfluidic diagnostics enable precise control of sample and reagent flow through micro-channels, allowing for the separation and detection of specific target analytes [59].

A key focus in the development of biosensor devices is on healthcare diagnostic tools. Recent advancements in this area include the integration of microfluidics and optical technologies, the miniaturization of devices, enhanced communication capabilities, and the introduction of simpler fabrication techniques. These innovations are driving the potential for future commercial applications of biosensors in healthcare diagnostics [4].

POC testing systems need varied sampling approaches and specific readout units. These platforms integrate diverse sensing mechanisms like electrochemical, colorimetric, fluorescent, and spectroscopy, targeting proteins, disease biomarkers, and cell count, for instance. The existing POC tests have strengths and weaknesses, highlighting the need for technological advancements to enhance analysis speed, sensitivity, miniaturization, and device affordability [59]. Obtaining blood samples from patients is a simple and common practice, making blood the primary medium for diagnostic testing across various health conditions [65, 66].

2.2.1.1 Point-of-care technologies in the cardiovascular field

Healthcare professionals specializing in cardiovascular care commonly integrate POC tests into their clinical routines. The utilization of cardiovascular-related POC is typically aimed at avoid-



Figure 2.7: POC Testing examples [64].

Table 2.1: Currently available point of care tests and the clinical utility/setting.

POC Device	Description
A1CNow+™ (Metrika, member of Bayer Healthcare LLC)	The improved performance of the A1CNow+ device enables swift hemoglobin A1C measurements, greatly enhancing diabetes management in clinical practice by providing accurate results in just 3 minutes [67].
HemoCue (Danaher)	This device is used to measure hemoglobin levels and exclude anemia. It is commonly utilized in operating theaters, clinics, general practices, and obstetrics [22].
Rotational thromboelastometry (ROTEM), Thromboelastography (TEG)	Viscoelastic assays, including rotational thromboelastometry and thromboelastography, are utilized in trauma and obstetrics settings. These tests are frequently performed in operating theaters and emergency departments [22].
Sonoclot system (Sienco)	The Sonoclot system (Sienco) is used in cardiothoracic theaters for monitoring heparin and assessing activated clotting time in cases of disseminated intravascular coagulation [22].
CareStart (CareStart™), Optimal (BioRad) and Clearview (Alere)	Malaria antigen tests are used for malaria screening and identification in laboratories and rural settings [22].

ing adverse health consequences, specifically preventing delays in identifying and treating time-critical conditions like myocardial infarction and stroke [68]. Some examples of POC technologies used in the cardiovascular field are shown in the Table 2.2, adapted from [4].

As previously mentioned, stroke is a medical emergency where time is crucial, and seeking early specialized care can significantly enhance outcomes. Recognizing a 'true stroke' during an emergency medical services call is difficult, with more than 50% of strokes being misclassified. Recent advancements offer new possibilities for diagnosing and treating strokes before a patient reaches the hospital. These include the incorporation of POC testing in ambulances [4].

There is a lack of a simple, immediate, and unbiased method for diagnosing stroke. Using POC tests as a bedside tool in the emergency department has, in some cases, led to faster medicine admission and improved outcomes for stroke patients [4].

Table 2.2: Stroke POCT Devices.

POC Device	Description
Mobile Stroke Unit (MSU)	Combining CT scanners with POC testing in ambulances allows for the immediate administration of IV-tPA treatment on-site. The pre-hospital team uses POC devices to assess coagulation profiles, perform complete blood counts, and analyze blood chemistry.
CoaguChek® (Roche)	The device, intended for monitoring oral anticoagulation therapy, uses test strips with electrochemical sensors to rapidly measure prothrombin time through amperometric detection.
Hemochron®Junior (ITC)	The system utilizes optical detection as a micro-coagulation system, delivering rapid results for coagulation parameters.
PocH-100i hematology analyzer (Sysmex)	The microfluidics-based system utilizes direct current detection with hydrodynamic focusing to count WBCs, RBCs, PLTs, and analyze hemoglobin.
i-STAT (Abbott)	The microfluidics-based system provides a fast and dependable diagnostic solution, delivering laboratory-quality results for parameters including blood gases, electrolytes, metabolites, and coagulation.
Reflotron®plus analyzer (Roche, Cobas series)	The system uses test strips with optical detection through reflectance photometry to conduct single-test clinical chemistry, measuring a range of blood chemistry parameters in whole blood, plasma, or serum.
Abbott AxSYM®BNP/Alere/Triage®BNP/i-STAT BNP	The system employs various detection techniques, including optical detection (AxSYM), fluorescent detection (Triage), and an electrochemical sensor on a silicon chip (i-STAT) for BNP POC testing.
Prediction Sciences LLC	Lateral flow POC testing for cellular fibronectin in stroke patients undergoing IV-tPA treatment helps assess the risk of potential hemorrhage.
ReST™(Valtari Bio™Inc.)	A rapid stroke triage POC test is performed to measure brain-specific biomarkers related to immune responses in the blood, aiding in the precise differentiation between acute stroke and non-stroke conditions.
SMARTChip(Sarissa Biomedical)	A micro-electrode POC testing device for stroke diagnosis detects purines from a small blood sample, enabling quicker identification of stroke patients and supporting stroke diagnosis in the hospital setting.
PFA-100®, Platelet Function Analyzer (Dade Behring)	A POC testing system with a high-shear force dynamic flow setup assesses platelet aggregation under conditions that simulate thrombus formation following vessel wall injury.
Ultegra-RPFA VerifyNow Aspirin®test (Accumetrics)	A POC testing system using optical detection evaluates platelet aggregation in whole blood through turbidimetry. This method is valuable in preventing the recurrence of a second stroke.

In a recent study, researchers compared the results obtained from POC testing with those from the central hospital laboratory. The findings showed that using POC testing, instead of the central laboratory, significantly reduced the time to initiate therapy from an average of 84 ± 26 minutes to 40 ± 24 minutes ($p < 0.001$). Additionally, most laboratory test results from POC testing closely aligned with those from the central hospital laboratory, except for partial thromboplastin time and international normalized ratio. Currently, commonly used POC devices are primarily designed for managing general emergency department patients and include assessments of coagulation parameters and blood chemistry. However, they are not specifically tailored for stroke patient care, highlighting the need for new POC devices designed to address the unique challenges of stroke management [4].

2.2.1.2 Advancements in point-of-care technology

POC devices are poised to revolutionize healthcare delivery by offering compact, portable, and user-friendly solutions for rapid and accurate diagnostics at the point of care. These devices alleviate the pressure on centralized laboratories, allowing patients to receive timely results without the need for extensive hospital visits. As chronic diseases and aging populations strain healthcare systems, the cost-effective and efficient nature of POC devices is becoming increasingly vital in addressing these challenges. POC devices are empowering healthcare professionals with real-time results, enhancing decision-making and minimizing the necessity for multiple appointments, thereby saving valuable time and resources. Moreover, the integration of POC devices with electronic health records and other healthcare technologies facilitates seamless access to patient data, promoting efficient care delivery and reducing the likelihood of medical errors [56].

Advancements in POC testing technologies are categorized into smartphone-based, paper-based, and fully automated lab-on-a-chip platforms [59]. These innovations offer cost-effective solutions for personalized healthcare and mobile medicine. However, to successfully translate these emerging technologies into commercially viable platforms, challenges hindering widespread adoption must be addressed. Cellphone platforms are becoming promising tools for the next generation of smart devices, empowering individuals with capabilities for preventive medicine, mobile healthcare, and telemedicine. This trend has increased the demand for POC devices, enabling patients to conduct health assessments and receive results remotely, enhancing both convenience and accessibility. The rising interest in personalized health monitoring through smart devices suggests a transformative potential for next-generation POC technologies. The vision is for a future where most people use POC devices for self-management of their health [56, 62, 69, 70].

Emerging POC tests must exhibit high sensitivity, specificity, user-friendliness, and deliver swift results. These advancements aim to facilitate POC diagnostics for a range of medical conditions [65].

2.3 Current state-of-the-art spectroscopy methods

2.3.1 Spectroscopy in the medical field

Optical spectroscopy techniques, such as fluorescence, Raman, and infrared spectroscopy, which are capable of identifying the biochemical composition of samples, have become a key focus in recent research. These methods are being actively investigated for their ability to detect neoplastic changes and differentiate between normal and cancerous tissues in various types of cancers, including oral, cervical, and breast cancers. Compared to traditional methods, these techniques offer greater objectivity, efficiency, and the potential for *in vivo* testing [58].

Over the years, vibrational spectroscopy techniques have been studied and refined as promising tools for disease diagnosis. These methods are appreciated for their high specificity and their capacity to detect a broad spectrum of molecular species. Numerous diseases induce molecular-level changes, altering the chemical composition or structure within biofluids, tissues, or cells.

This ability is vital not only for histopathology, cytology, and pharmaceutical research but also for identifying specific cancers and viruses. As a result, spectroscopic techniques are particularly well-suited for detecting a wide range of disease states [65, 71].

In oncology, pathologists traditionally assess tissue samples to determine the stage and grade of a tumor. This approach is typically slow and subjective, focusing on morphological features in histological samples while neglecting the biochemical information at the cellular level. In contrast, vibrational spectroscopy techniques offer a biochemical fingerprint of the sample. Numerous proof-of-concept studies have shown that these techniques can evaluate and diagnose tissue samples much faster than conventional methods [71].

In the field of microbiology, vibrational spectroscopy techniques play a crucial role in quickly detecting and identifying microbes with exceptional sensitivity and selectivity. This is particularly challenging in various sectors, notably in clinical diagnostics, pharmaceuticals, and food processing technology. These non-invasive methods offer insights into molecular fingerprints, allowing for the quick and dependable analysis of intricate biological systems like bacterial cells and yeasts [58].

Spectroscopy offers several advantages for diagnostics using blood samples, particularly with its potential to be adapted for POC diagnostic devices. Infrared spectroscopy (IR), especially when combined with multivariate and machine learning algorithms, shows promise in meeting this need due to its minimal sample preparation requirements. Spectral data can be obtained using different types of equipment, from advanced infrared microscopes linked to synchrotron sources to portable attenuated total reflection spectrometers, which are becoming more popular as POC diagnostic tools. A spectrum is generated by passing an IR beam through a sample; the sample absorbs some of the light, while the rest reaches a detector. The recent development of IR spectroscopy for disease diagnostics includes the introduction of handheld Fourier-transform infrared spectroscopy devices that can analyze different types of samples [65].

Fluorescence microscopy and time-resolved fluorescence spectroscopy have proven effective in identifying and distinguishing various types of cancer and neoplastic changes too [72]. A novel and promising technique, Laser-Induced Fluorescence Spectroscopy (LIFS), is emerging as a diagnostic tool for tissue pathologies. Past research has demonstrated the utility of markerless steady-state LIFS in diagnosing brain neoplasms by analyzing the autofluorescence of endogenous fluorophores. This technique has been applied to the examination of different brain tumor types, both *in vivo* and *ex vivo*, including glioblastoma, astrocytoma, oligodendroglioma, and metastatic carcinoma [73].

2.3.1.1 Spectroscopy in the cardiovascular field

In the cardiovascular area, spectroscopy techniques are demonstrating encouraging outcomes in imaging vulnerable plaques. The coming years will reveal whether these techniques can effectively identify unstable cardiovascular disease. Ongoing clinical trials seek to validate whether the detection of a vulnerable plaque through intravascular spectroscopy increases the likelihood of causing cardiovascular events [58].

In summary, all the discussed spectroscopy techniques exhibit the potential to offer clinically significant diagnostic insights. Timely detection plays a vital role in preventing diseases. When coupled with advanced chemometrics, these measurements can play a valuable role in the diagnostic assessment of biological materials [58].

A collaborative effort is now required, bringing together clinicians, instrument developers, industry partners, health economists, and healthcare decision-makers, to ensure that the technology achieves its full potential and is effectively integrated into clinical practice [71].

2.4 Current state-of-the-art spectral point-of-care technologies

Several spectral technologies are currently under investigation for their application in POC devices. Spectroscopy stands out due to its versatility, as it doesn't require specific sample volumes or the use of reagents, making it well-suited for portable POC technology [33].

2.4.1 Absorption spectroscopy

POC device using visible near infrared spectroscopy (Vis-NIRS)

Visible-shortwave-near-infrared (Vis-SWNIR) spectroscopy using artificial intelligence emerges as a promising avenue for POC diagnostics due to its capacity to deliver comprehensive clinical analysis without needing reagents or intricate sample preparation. It captures blood spectra in real-time, offering a plenty of clinical parameters swiftly and efficiently. A specialized Vis-SWNIR spectral analysis POC system utilizing an artificial intelligence approach named Self Learning Artificial Intelligence (SLAI) has been developed specifically for assessing red blood cells (RBC), hemoglobin (Hgb), and hematocrit (HTC) levels in blood samples from dogs and cats, requiring just a drop of blood (<10 μ L) [33, 37].

This system has demonstrated significant potential as a POC technology for not only analyzing red blood cells, hemoglobin and hematocrit in feline and canine samples, but also for white blood cells (WBC) quantification, PLT quantification and diagnosing thrombocytosis in canine blood samples.

A feasibility study was conducted to directly detect white blood cell counts in canine blood using visible-near infrared spectroscopy. The prototype Vis-SWNIR point-of-care IoT device, which employs UV-Vis spectroscopy and a Self-Learning Artificial Intelligence (SLAI) method, provided a practical solution for analyzing WBC counts in canine blood samples. This technology delivers real-time results from just a single drop of blood [38].

Through innovative spectroscopy analysis and a novel artificial intelligence processing approach, an exploratory research showcases the feasibility of thrombocyte quantification directly in dog whole blood. These findings suggest the potential of spectroscopy as a diagnostic tool for swiftly detecting elevated platelet levels, thereby aiding in the rapid diagnosis of thrombocytosis and facilitating stroke prevention [33].

Point-of-care using near infrared spectroscopy (NIRS)

A research has investigated the integration of two techniques to evaluate stroke and brain health. The goal is to enable affordable POC assessment of cerebral vascular status. Cerebrovascular reactivity (CVR) is crucial for assessing cerebral vascular status, reflecting blood vessel dilation capacity and serving as a marker for brain vascular reserve. Studies indicate that impaired cerebral hemodynamics, as indicated by reduced CVR, precedes strokes and transient ischemic attacks (TIA). CVR provides important insights beyond standard baseline blood flow measurements, as a significantly reduced CVR can indicate a higher risk of stroke and transient ischemic attack (TIA). Moreover, cerebral blood flow is strongly linked to neural activity, with glucose being delivered through neurovascular coupling. Near-infrared spectroscopy (NIRS) allows for continuous monitoring of cerebral oxygenation and blood volume, capturing changes in blood flow associated with neural activity. Additionally, cortical neural activity can be influenced using transcranial direct current stimulation (tDCS) [5].

NIRS is a non-invasive technique that uses light to assess blood flow and oxygen levels in the brain, particularly cerebral hemoglobin oxygenation. It is widely used to monitor cerebral vascular status in various clinical settings due to its safety and portability, making it ideal for POC testing. Transcranial direct current stimulation (tDCS) involves applying mild electrical currents to the scalp to alter brain activity. In this study, tDCS is used to induce changes in cerebral blood flow [5].

Researchers developed a portable device that combines NIRS and tDCS using an adaptive neuro-fuzzy inference system (ANFIS) implemented in field-programmable gate array based hardware. This device aims to assess cerebrovascular reactivity, which indicates how well blood vessels in the brain dilate in response to anodal tDCS stimulation. ANFIS, an advanced artificial intelligence algorithm, interprets the combined NIRS and tDCS data to potentially provide stroke risk assessment for POC testing. The researchers conducted a study involving 14 patients who recently experienced ischemic strokes, comparing CVR between the affected side of the brain and the healthy side. They CVR before and after applying tDCS were assessed. The stroke-affected side of the brain exhibited a notably smaller rise in blood oxygenation following tDCS, compared to the healthy side. This indicates compromised CVR in the brain tissue affected by the stroke. The findings suggest promising outcomes for the novel tool employing NIRS-tDCS in distinguishing between stroke-affected and healthy brain areas based on their reaction to stimulation. The integration of an AI system (ANFIS) holds potential for automating this analysis, offering a more objective and streamlined approach to stroke assessment [5].

Another investigation also focused on a low-cost wearable NIRS device for detecting ischemic stroke conditions. By incorporating new hemodynamic biomarker data, the device offers an alternative POC solution for early detection of ischemic conditions, potentially reducing mortality rates by enabling timely intervention. NIRS presents a promising alternative to conventional methods like MRI and CT scans, which are costly and lack portability. Unlike these methods, NIRS is

non-invasive and portable, making it suitable for various settings. Additionally, NIRS measures hemodynamic activity, providing valuable insights into blood flow dynamics [74].

This solution aims to overcome the limitations of existing NIRS devices, such as high cost and limited data collection capabilities. By introducing a multichannel, multiwavelength, and multibiomarker-based POC NIRS system, this approach offers a more affordable alternative compared to previous studies. The incorporation of additional biomarkers enhances the physiological information available for assessing ischemic tissue conditions, surpassing the capabilities of existing devices with fewer biomarkers. The researchers simulated stroke conditions by inducing restricted blood flow at different stages. In summary, the NIRS device introduced in this research presents a promising solution for stroke detection, especially in settings with limited resources. Its cost-effectiveness, utilization of multiple biomarkers, and validation through established techniques highlight its potential as a valuable instrument for early stroke diagnosis, potentially leading to better patient outcomes [74].

2.4.2 Vibrational spectroscopy

Point-of-care device using surface enhanced raman spectroscopy (SERS)

A promising application of spectroscopy in POC devices is a novel method for analyzing neurotransmitters directly in whole blood samples at the point of care. This innovative approach leverages Surface-enhanced Raman spectroscopy (SERS) within a device designed to detect neurotransmitters in blood samples. Neurotransmitters, which are chemicals released by various cells in the body, are crucial for transmitting signals between nerve cells and other target cells, such as muscles and glands. Variations in neurotransmitter levels in bodily fluids are closely linked to mental disorders, neurodegenerative diseases, and cardiovascular conditions. Detecting these neurotransmitters aids in diagnosing clinical conditions and provides a deeper understanding of the biological mechanisms underlying these disorders. Traditional methods for detecting neurotransmitters often require complex procedures and lengthy processing times. Consequently, SERS has become a valuable tool for POC biochemical analysis due to its high sensitivity and fast results. SERS enables the rapid and direct identification of target molecules without the need for additional labeling, making it a versatile technique widely used in various fields, such as food safety, environmental monitoring, disease diagnosis, and drug analysis. The developed device successfully measured the neurotransmitter dopamine with high accuracy. Given its ease of production, user-friendliness, rapid results, and cost-effectiveness, this device is expected to have broad applications in diagnosing and monitoring diseases directly at the point of care [75].

Numerous novel POC approaches utilizing Raman systems have been investigated. However, the focus has predominantly been on developing assays, with insufficient attention given to understanding the essential features necessary for Raman spectrometers to effectively operate in POC settings. A research investigated the practicality of utilizing SERS systems as POC platforms. It assessed the effectiveness of eight different commercial Raman spectrometers, each coupled with

a paper fluidic cartridge, for detecting biomarkers associated with cardiovascular disease. Specifically, the focus was on evaluating their capability to detect cardiac troponin I and heart fatty acid binding protein, which are widely recognized biomarkers used in assessing cardiovascular health. The spectrometers used in the study varied in size, ranging from larger benchtop Raman microscopes to more portable and handheld versions. The paper fluidic cartridges for surface-enhanced Raman spectroscopy were fabricated using a method established previously. These cartridges demonstrated significant sensitivity in detecting cardiac troponin I (cTnI) and heart fatty acid binding protein within the clinically relevant range. The study revealed that the detection sensitivity of portable and handheld spectrometers was comparable to that of the standard laboratory instrument. This suggests that portable Raman spectrometers hold promise for POC applications, offering accuracy akin to high-end laboratory devices. Moreover, they are cost-effective, provide rapid signal readouts, are user-friendly, and could enhance healthcare access for marginalized populations [61].

Another study using the SERS method introduces a novel approach for detecting cardiac biomarkers in acute coronary syndrome. This method employs a nanofluidic biosensor equipped with a microchannel that narrows into a nanochannel, where gold nanoparticles aggregate to form SERS-active sites. The capillary flow facilitates the aggregation of these nanoparticles, enhancing electromagnetic fields and shifting the plasmon resonance toward the near-infrared spectrum. This innovative technique shows great potential for developing a POC diagnostic system for acute coronary syndrome. Researchers have successfully utilized SERS to qualitatively detect key biomarkers such as brain natriuretic peptide, troponin I, and C-reactive protein. SERS has proven to be a dependable method for protein analysis, outperforming other techniques like infrared spectroscopy, circular dichroism, nuclear magnetic resonance spectroscopy, and X-ray diffraction. This approach effectively addresses the challenge of limited areas with enhanced electromagnetic or plasmonic activity. While current assays for detecting cardiac biomarkers often rely on sandwich enzyme-linked immunosorbent assays, this new method introduces SERS as a way to overcome this limitation. The nanofluidic biosensor operates by mechanically trapping the target molecule between clusters of gold nanoparticles at the junction of the nano-microchannel, fostering plasmonic interactions with the biomolecule at a specific site [21].

The distinct patterns of peaks observed in the SERS spectra for each biomarker enable their identification. Through careful analysis of these prominent peaks, researchers can elucidate the specific interactions between each biomarker molecule and the gold nanoparticles within the sensor. Additionally, the spectra offer valuable insights into the structural characteristics of the proteins. The findings indicate the promising potential of the SERS-based nanofluidic biosensor for detecting and distinguishing between various cardiac biomarkers. However, the relatively weak nature of ionic bonding may lead to the easy adsorption and desorption of molecules from the surface. As a result, future research efforts will be directed toward optimizing the assay for quantitative information [21].

2.4.3 Electromagnetic spectroscopy

Point-of-care device using dielectric spectroscopy

Another notable example is the ClotChip, a microfluidic sensor designed for dielectric spectroscopy analysis of human whole blood during coagulation. The ClotChip shows promise as a POC platform that can evaluate the entire hemostatic process using less than 10 microliters of blood. Timely assessment of coagulation and platelet function is vital for managing patients in critical conditions, such as those with severe injuries, undergoing surgery, or receiving antiplatelet or anticoagulant therapies. In these scenarios, healthcare providers must make rapid decisions about treatment, transfusion strategies, or maintaining appropriate anticoagulant levels. Current coagulation tests are usually conducted in centralized laboratories by specialized personnel, which often results in delays due to the time required to obtain results, potentially postponing crucial information on hemostatic imbalances. Dielectric spectroscopy provides a simple and non-invasive approach to measure the physiological properties of blood in real-time using only electrical signals, eliminating the need for labels or destructive processes. This makes it ideal for developing easy-to-use POC devices that can quickly provide valuable insights into blood physiology. The ClotChip has the potential to become an affordable, compact, disposable microfluidic sensor, enabling rapid and comprehensive diagnosis of blood coagulation and platelet disorders at the point of care [76].

Point-of-care device using surface plasmon resonance (SPR) spectroscopy and fluorescence spectroscopy

In a research study, scientists developed and evaluated a Surface Plasmon Field-Enhanced Fluorescence Spectroscopy (SPFS) device featuring a convergent optical system. This instrument demonstrated high sensitivity in detecting low levels of alpha-fetoprotein (AFP) in fetal bovine serum samples. SPFS shows great potential as a method for developing a POC device with outstanding sensitivity. It utilizes the intensified optical field generated by a surface plasmon mode to excite fluorophores located near a metal-dielectric interface [77].

Yu and colleagues developed a sensing device using SPFS technology, achieving remarkable sensitivity in detecting free prostate-specific antigen [28].

This research demonstrated the quantitative detection of AFP, a marker for liver cancer, at levels below the clinical test cutoff threshold. Both theoretical models and AFP immunoassays indicated that the SPFS device maintained stability despite changes in the refractive indices of the samples. These results are important for the development of compact and user-friendly measurement devices with high sensitivity and accuracy [77].

POC measurements for highly sensitive cardiac troponin (hs-cTn) assays are currently not available due to a performance gap between central laboratory systems and existing POC tests. Despite analytical improvements in cTn assays, current POC options still lack the necessary sensitivity. Rapid testing should not compromise clinical accuracy, emphasizing the urgent need for the

development and thorough validation of POC assays for highly sensitive cardiac troponin. Surface plasmon fluorescence spectroscopy (SPFS) is recognized for its exceptional detection sensitivity due to its minimal background noise. In a study conducted by Kaya et al., SPFS was effectively used to detect low levels of prostate-specific antigen in serum using a plastic sensor chip. Their findings indicate that SPFS could be a promising technique for detecting low concentrations of biomarkers, such as cTn, in clinical samples [78].

Konica Minolta (KM) has created a POC system utilizing surface plasmon fluorescence spectroscopy (SPFS) that shows great promise for delivering high-quality hs-cTn I results. This innovative system is designed to uphold performance standards comparable to those of central laboratories, providing reliable results without compromising accuracy or sensitivity. The KM SPFS POC system has demonstrated satisfactory performance in measuring cTnI levels in both whole blood and plasma samples. However, some limitations should be taken into account when interpreting these results [78].

2.5 Artificial intelligence

Artificial intelligence (AI) involves the scientific and engineering efforts to create machines that can imitate human cognitive functions, especially in areas like learning and problem-solving. The main goal of AI is to replicate human cognitive skills, enabling machines to carry out tasks that usually require human intelligence, such as making decisions and recognizing patterns [79].

2.5.1 Artificial intelligence in the medical field

AI is transforming the healthcare industry by improving patient outcomes and lowering costs. It is essential in numerous areas of healthcare, such as medical imaging, diagnostics, pandemic management, virtual patient care, treatment compliance, and handling administrative tasks. Despite these advancements, several challenges persist, such as data security, privacy, legal concerns, and the inherent limitations in data quality and the ability of AI to capture the complexity of human traits. Nevertheless, the potential benefits of AI in healthcare are substantial, driven by the increasing availability of health data and rapid advancements in analytical technologies. Globally, healthcare systems are under pressure due to rising costs that outpace GDP growth, further strained by the COVID-19 pandemic and geopolitical conflicts like the war in Ukraine. These factors, combined with financial constraints, an aging population, and the growing prevalence of chronic diseases, are pushing healthcare systems to their limits. In response, breakthroughs in biomedical science, genomics, digital medicine, and AI, particularly in machine learning (ML), are paving the way for a transformation in healthcare. This transformation necessitates the development of a new workforce equipped with the skills to utilize these technologies and the establishment of higher standards of practice. Moreover, it is crucial that AI development is governed in a manner that aligns with public interests, addressing not only technical aspects but also ethical and social considerations [79]. The figure 2.8 is showing a scheme representing the role of AI in healthcare.

In the healthcare sector, disease prediction is significantly enhanced by data mining, which plays an essential role in extracting valuable insights from the vast amount of medical data generated by hospitals. Patient records, a growing source of medical data, contain valuable information that, when analyzed effectively, can be transformed into a substantial resource for medical research and improving patient care [80, 81].

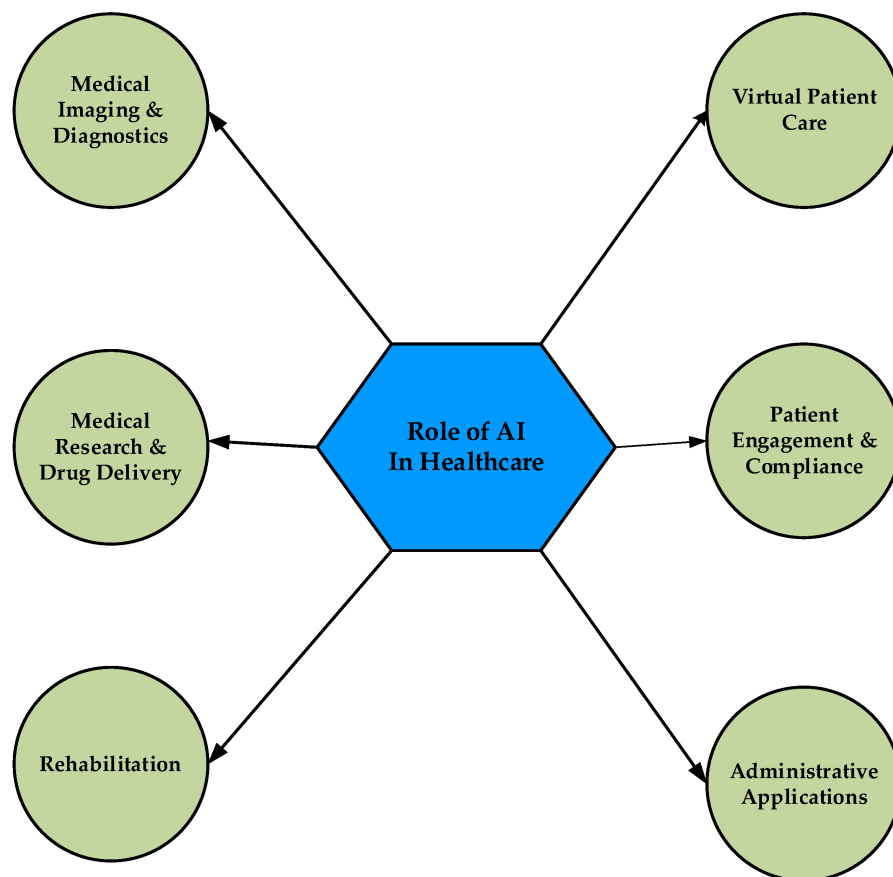


Figure 2.8: Role of AI in Healthcare [79].

2.5.1.1 Artificial intelligence in the cardiovascular field

AI has made significant strides in the cardiovascular field, particularly in the diagnosis and management of stroke. One notable development is the creation of a predictive model using an Artificial Neural Network (ANN) to improve existing diagnostic methods for thromboembolic strokes. This ANN was trained using the backpropagation algorithm and tested across various stroke categories. The results demonstrated an 89% improvement in diagnostic accuracy when using the ANN-based approach compared to traditional methods [82].

Another stroke prediction model was created using classification algorithms like Decision Trees, Naïve Bayes, and Neural Networks to predict the occurrence of the disease. Additionally, Principal Component Analysis (PCA) was applied to reduce data dimensionality, improving

the model's overall performance. The results demonstrated that the neural network surpassed Decision Trees and Naïve Bayes in accuracy, underscoring its superior ability to make predictions [83].

AI is also being utilized to aid medical decision-making in stroke care, especially in post-operative situations. For instance, deciding the most effective patient transfer strategy following surgery is a crucial part of medical practice. To support clinicians in making these decisions, classification techniques such as Support Vector Machines and Artificial Neural Networks (ANNs) have been suggested. These approaches are designed to enhance the accuracy and efficiency of post-operative decisions, thereby improving patient outcomes [20].

Chapter 3

Methodology

This chapter presents the main materials, experimental and statistical methods used throughout the process of testing the implementation of the POC analysis device.

The methodology involved the use of a POC device prototype developed at INESC TEC, which functions as a cellphone plug-in. These optical devices are designed to capture the spectra of whole-blood samples using a power LED as the light source. The light interacts with the blood, and the transmitted or reflected spectrum is captured. A small volume of blood is sufficient for spectral data acquisition, making the device practical for POC testing. The device is user-friendly, with controls and data accessible through an Internet of Things interface on a computer or smartphone. The low-cost components, portability, and ease of use make these devices suitable for various settings. Figure 3.2 illustrates a scheme of the procedure using the POC device.

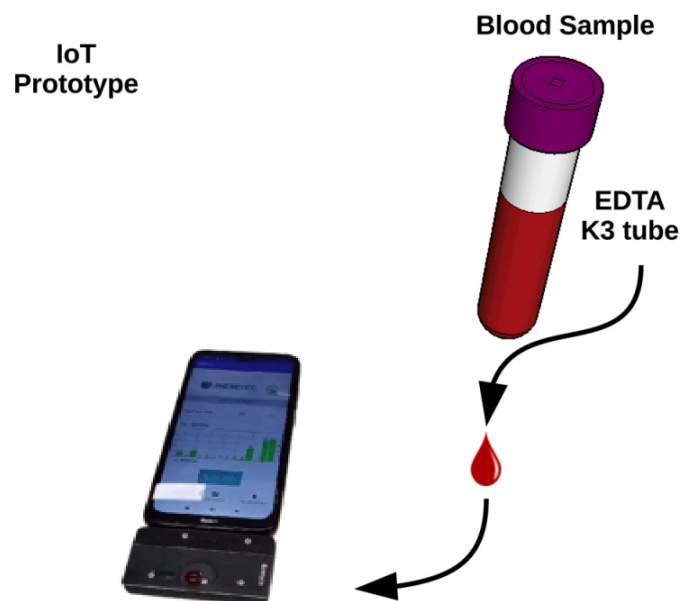


Figure 3.1: Scheme of procedure. [37]

Blood spectra were obtained using the POC device, which comprised a power LED and a miniaturized USB-based spectrometer. Blood samples were drawn from patients by trained professionals at the clinical analysis department of the Vila Nova de Gaia/Espinho Hospital Center, following standardized venipuncture procedures. The samples were stored in K3-EDTA tubes and analyzed within 8 hours to ensure their quality and integrity. In the laboratory, after undergoing the hospital's routine laboratory analysis procedures, the blood samples were gently mixed to ensure consistency before spectral acquisition. Next, around 100 μL of each blood sample were carefully drawn from the blood count tube using a pipette and placed onto a glass slide positioned in contact with the device's LED light source. The LED light illuminated the sample, and the spectrometer captured the light spectra, that were then saved on a computer.

The POC device, which was connected to a smartphone via a plug-in, used an IoT platform via USB, enabling real-time monitoring and data recording through a specialized smartphone application. The IoT platform communicated wirelessly with the *RStudio* program on a computer, where a database of the spectral data was maintained. The entire procedure was performed under low light conditions to prevent any external light from affecting the results.

A total of 312 blood samples were analyzed, and spectroscopic signals were recorded at three different wavelengths, spanning from 300 to 800 nm, which covers the ultraviolet, visible, and infrared regions. The data was divided into two categories: blood count data and spectroscopy data, both of which were associated with an anonymized key to ensure patient confidentiality. The complete blood count (CBC) dataset included quantitative information on various parameters such as red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC) and total platelet count (PLT).

Hematocrit represents the percentage of red blood cells within the total blood volume. It indicates the proportion of blood composed of red blood cells. This measure is useful for evaluating the blood's ability to transport oxygen and can assist in diagnosing conditions like anemia or dehydration.

The main goal was to demonstrate the reliability of the POC device for quantifying blood cells in humans. To achieve this, the spectrum measurements obtained were related to the cell count in the blood and the data obtained was compared to the quantifications performed by the hospital's routine equipment, the Sysmex XN-1000™, to validate the accuracy and reliability of the POC device in blood cellular quantification.



Figure 3.2: POC device connected to a smartphone with the Iot platform represented, showing an example of a blood cell count results and its graphs.

The equipment Sysmex XN-1000™ uses three different methods [84]:

- Fluorescent flow cytometry for differential WBC counting, reticulocyte counting and PLT counting with fluorescence.
- Impedance with hydrodynamic focusing for RBC counting and PLT counting with impedance.
- Sodium lauryl sulphate, cyanide-free for HGB counting.



Figure 3.3: Sysmex XN 1000. [84]

3.1 Data curation

Data curation is essential due to the presence of experimental errors in both automated hematology and spectroscopy measurements. Abnormal relationships in automated hematology data, such as incorrect correlations between RBC, Hgb, and HTC, or unusually low PLT values being mistakenly counted as WBCs or RBCs, were removed from the dataset to eliminate experimental artifacts. Spectral data were also curated to exclude records that were either too low or saturated, as these do not follow detector linearity.

3.2 Principal component analysis

Principal Component Analysis (PCA) was applied to both the blood count composition and spectral data to examine the variance structure within these datasets. PCA identifies the eigenstructure of a dataset by maximizing its variance, providing an independent analysis on how the information of each dataset is structured. If the information structures are similar but expressed in different units or scales of variation, this suggests that both datasets capture the same underlying information, but on a different basis. This serves as an initial indication that a direct relationship can be established between spectral features and hemogram parameters, ensuring both global and local covariance between the two independent datasets.

3.3 Chemometric method

3.3.1 Partial least squares

Partial Least Squares (PLS) is a statistical technique that identifies relationships between two data sets: predictor variables and response variables. In this study, PLS regression is employed to link spectral data from a POC device with blood cell counts obtained from a Sysmex machine. PLS reduces the complexity of the spectral data to accurately predict blood cell counts, thereby offering a model to assess the effectiveness of the POC device in measuring these counts [85].

Partial least squares maximizes the covariance between the spectra matrix $\mathbf{X}$ and blood composition matrix $\mathbf{Y}$, by identifying the orthogonal eigenvectors of the covariance matrix $\mathbf{X}'\mathbf{Y}$ [85, 86]. This method ensures that the latent structures of the spectra and composition ($\mathbf{t}$ and $\mathbf{u}$) are equal, as determined by the NIPALS algorithm, to establish each corresponding basis $\mathbf{P}'$ and $\mathbf{Q}'$, where:

Partial Least Squares (PLS) aims to maximize the covariance between the spectra matrix $\mathbf{X}$ and the blood composition matrix $\mathbf{Y}$, by identifying the orthogonal eigenvectors of their covariance matrix, $\mathbf{X}'\mathbf{Y}$ [85, 86]. This approach ensures that the latent variables of both the spectra and the composition ($\mathbf{t}$ and $\mathbf{u}$) are matched, as determined by the NIPALS algorithm, to define each corresponding basis $\mathbf{P}'$ and $\mathbf{Q}'$, where:

$$\mathbf{X} = \mathbf{TP}'$$

$$\mathbf{Y} = \mathbf{UQ}'$$

The procedure continues with deflation and the sequential calculation of orthogonal eigenvectors for the remaining data in the covariance matrix $\mathbf{X}'\mathbf{Y}$. The optimal number of latent variables (LV) is determined by minimizing the predicted residual sum of squares (PRESS) through cross-validation. PLS uses an oblique projection to determine the coefficients $\mathbf{b}_{pls}$ in the model represented by the equation $\mathbf{Y} = \mathbf{X}\mathbf{b}_{pls}$ [86, 87].

3.4 Artificial intelligence method

3.4.1 Self-learning artificial intelligence

Self-learning artificial intelligence (SLAI) was created to address the complex challenge of analyzing interference patterns in spectroscopy data when examining blood samples. This technology turns these interferences into an advantage by finding consistent relationships, known as covariance, between blood composition and spectral features. This method has been patented by INESC TEC under the Center for Applied Photonics, affirming its innovative approach to handling complex biological data. This patent (Patent WO2018060967) highlights that the unique method SLAI offers to the scientific community, promising significant advancements in the field of medical diagnostics and beyond [88].

The core idea behind SLAI is its ability to identify stable patterns within the data, which helps to unscramble the complex interferences that happen when different blood components overlap in the spectral measurements. This process is called Covariance Mode (CovM) search, where SLAI isolates specific interference patterns in the spectral data. By doing this, SLAI can accurately extract quantitative information about the blood's composition [37].

In simpler scenarios, such as with pure substances, the relationship between the spectral data and the substance's concentration is direct and straightforward because there are no overlapping interferences. However, in more complex biological samples like blood, the spectral data is spread across many wavelengths due to overlapping spectral bands and other effects like pH variations or light scattering. This makes the global covariance unstable and multi-dimensional. SLAI addresses this complexity by separating and analyzing these interferences to accurately quantify each blood component. An optimal CovM isolates a group of samples that share similar interference patterns, enabling SLAI to establish a direct relationship between the spectral data and blood composition. Ideally, this relationship is represented by a single latent variable, meaning the patterns in the spectral data closely match those in the composition data. This allows for a clear understanding of how spectral features relate to blood composition, improving the model's ability to predict the composition of new samples. Additionally, CovM is closely linked to the baseline levels of each blood constituent, making it a powerful tool for accurately interpreting and predicting blood composition from complex spectral data. Furthermore, the ability of SLAI to identify which covariance mode, or combination of modes, best fits an unknown sample in the feature space allows for precise quantitative measurements. This capability is crucial for advancing diagnostic techniques in medical settings, providing a robust method for analyzing blood samples with high precision [33, 37].

In summary, SLAI operates by detecting stable covariance patterns within spectral datasets and identifying CovM, which consists of samples with similar interference characteristics. This approach enables SLAI to create a proportional link between cell quantification and spectral features, ideally captured by a single eigenvector or latent variable (LV). The reliability of CovM is confirmed using cross-validation methods, such as leave-one-out, to ensure consistent results.

The two statistical methods used different metrics to evaluate their performance, comparing variables related to the main blood cells (RBC, WBC, and PLT), as well as hemoglobin (HGB) and hematocrit (HTC). Each method's effectiveness was assessed by calculating the standard error (SE), mean absolute percentage error (MAPE), and Pearson correlation coefficient (R) to gauge the linearity between predicted and actual values. These metrics were determined both within and outside the reference interval (RI). All models were constructed using median spectra and validated with leave-one-out cross-validation. All computations were performed using the *R Studio* as statistical computing software (PLSR package and SLAI using the author's code).

SE measures the accuracy with which a sample represents a population. In the context of this study, it quantifies the average error in the predictions made by the model compared to the actual

measured values. A lower SE indicates a model with predictions closer to the observed data [89].

Standard error (SE):

$$SE = \frac{s}{\sqrt{n}}$$

s - Standard deviation of the sample

n - Total number of observations

The MAPE is a measure of prediction accuracy of a forecasting method in statistics. It expresses the difference between actual and predicted values as a percentage, providing a simple interpretation in terms of relative error. It is particularly useful because it scales the error to the size of the items being forecast, making the results easier to understand and compare. A lower MAPE value indicates a better performance of the model, with 0% being the ideal [89].

Mean absolute percentage error (MAPE):

$$MAPE = \frac{100}{n} \sum_{i=1}^n \left| \frac{y_i - \hat{y}_i}{y_i} \right|$$

y_i - Actual value for observation _{i}

$\hat{y}_i$ - Predicted value for observation _{i}

n - Total number of observations

Pearson's correlation coefficient (R) quantifies the linear relationship between two data sets. Here, it is used to evaluate the degree of linearity between the predicted values generated by the model and the actual measured values of blood components. The coefficient ranges from -1 to 1, where 1 signifies a perfect positive linear correlation, 0 indicates no linear relationship, and -1 represents a perfect negative linear correlation. A larger absolute value of R denotes a stronger correlation between the two sets of data [89].

Pearson's correlation coefficient (R):

$$R = \frac{\sum_{i=1}^n (y_i - \bar{y})(\hat{y}_i - \bar{\hat{y}})}{\sqrt{\sum_{i=1}^n (y_i - \bar{y})^2} \sqrt{\sum_{i=1}^n (\hat{y}_i - \bar{\hat{y}})^2}}$$

y_i - Actual value for observation _{i}

$\hat{y}_i$ - Predicted value for observation _{i}

$\bar{y}$ - Mean of the actual values

$\bar{\hat{y}}$ - Mean of the predicted values

n - Total number of observations

Chapter 4

Results and Discussion

This chapter outlines the findings from the evaluation of the POC device, comparing its performance with that of standard hospital blood cell counting equipment. It also includes a discussion of these results.

One of the aims of this study was to evaluate and validate the performance of a POC device by comparing it with the Sysmex XN 1000, a highly advanced hematology automated equipment used for analysing blood cells. It is crucial to recognise that although the POC device conforms to the technical capabilities of the Sysmex, it is still a prototype under continuous development and is not yet a finished product. This process of continuous development is fundamental to perfecting the device's specifications.

This research aims to improve and provide more effective tools to support healthcare professionals, focusing on the potential of POC systems to manage matrix effects and interference at various scales, thus advancing the capabilities of reagent-less optical biosensors in clinical settings.

The scatterplot matrix presented in Fig. 4.1 is the dataset characterisation. The natural correlation between RBC, Hgb, and HTC is expected as larger numbers of RBC imply higher concentrations of Hgb and larger volume of RBC in blood (HTC). The correlation extends to the derived hemogram parameters mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH). The existing natural correlation is commonly known in hematology as the rule of three, where : $Hgb = 3 \times RBC$ and $HTC = 3 \times Hgb$. Methods using similarity optimization metrics (e.g. SVM, ANN, or DL), and variance maximization (e.g. PCR), and correlation (e.g. LocPLS and PLS) will follow the rule of three, not unscrambling RBC size and hemoglobin distributions [37, 38].

LEU and PLT parameters do not exhibit strong linear relationships with any of the other variables, indicating that they vary independently within the dataset and they might be influenced by different factors. This matrix offers a clear visual representation of the interrelationships among these variables, which is valuable for detecting patterns or potential multicollinearity before performing statistical analyses or modeling.

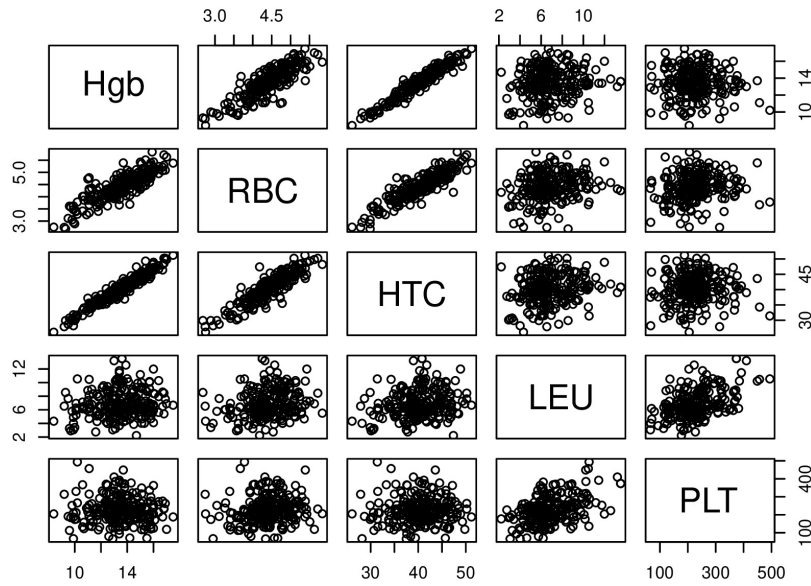


Figure 4.1: Correlation Plot

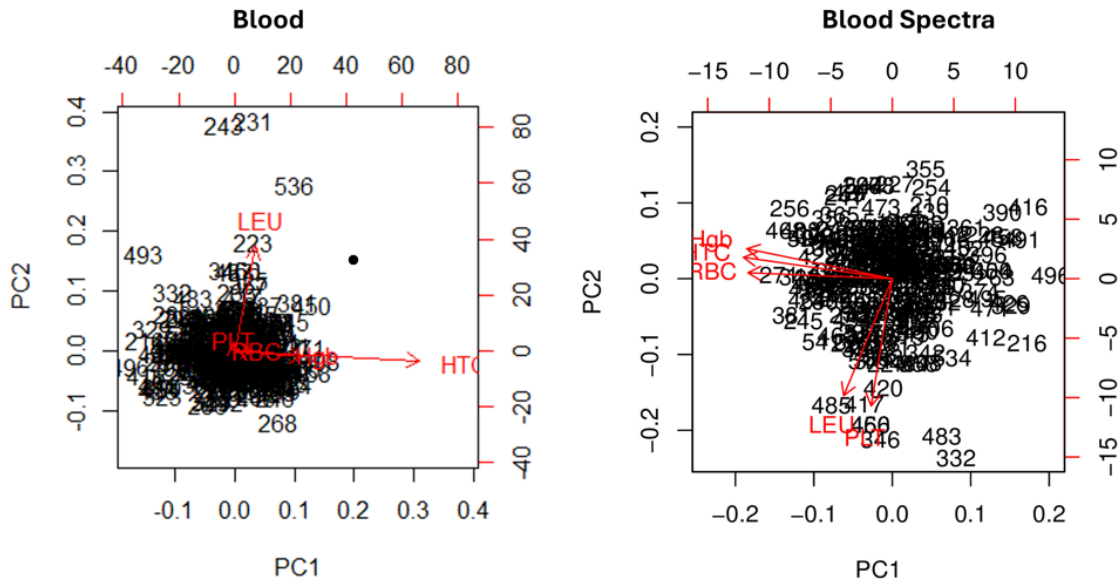


Figure 4.2: PCA

The Figure 4.2 present the results of principal component analysis (PCA) applied to both hemogram and spectral data. The PCA graphs are consistent with the correlation plot, clearly illustrating the relationships among the variables.

In this study, the spectral data ($\mathbf{X}$) and hemogram data ($\mathbf{Y}$) are decomposed into principal components: $\mathbf{X} = \mathbf{TP}^T$ for spectra and $\mathbf{Y} = \mathbf{UC}^T$ for hemogram. If the resulting transformed components ($\mathbf{T}$ and $\mathbf{U}$) display similar patterns, it suggests that both datasets convey shared underlying information. This allows for a more effective comparison and analysis of the data.

For the hemogram data PCA, the main dominant loadings are hematocrit (HTC) and leucocytes (LEU), exhibiting a negative correlation. PC1 is primarily driven by variations in HTC, RBC and Hgb, while PC2 is primarily influenced by variations in LEU and PLT. HTC and RBC eigenvectors are close to each other, indicating a strong positive correlation between these two parameters. LEU and PLT are positioned nearly orthogonally to HTC, RBC and Hgb, indicating a weak or no correlation between these parameters. This separation along the principal components may indicate that HTC and RBC are related and vary together, whereas PLT and LEU represent different patterns or subgroups within the data.

In the spectral data PCA, Hgb, RBC and HTC seem to be the primary contributors to PC1 (first principal component), whereas LEU and PLT have a more significant influence on PC2 (second principal component). Based on the variable contributions to PC1 and PC2, it might be inferred that variations in Hgb, RBC, and HTC are more closely related to each other than to LEU and PLT. The clustering of points suggests that there might be distinct subgroups or patterns within the data. A few points seem to be outliers, potentially representing unusual or extreme data points. The outliers might represent individuals with unusual or extreme values for one or more variables.

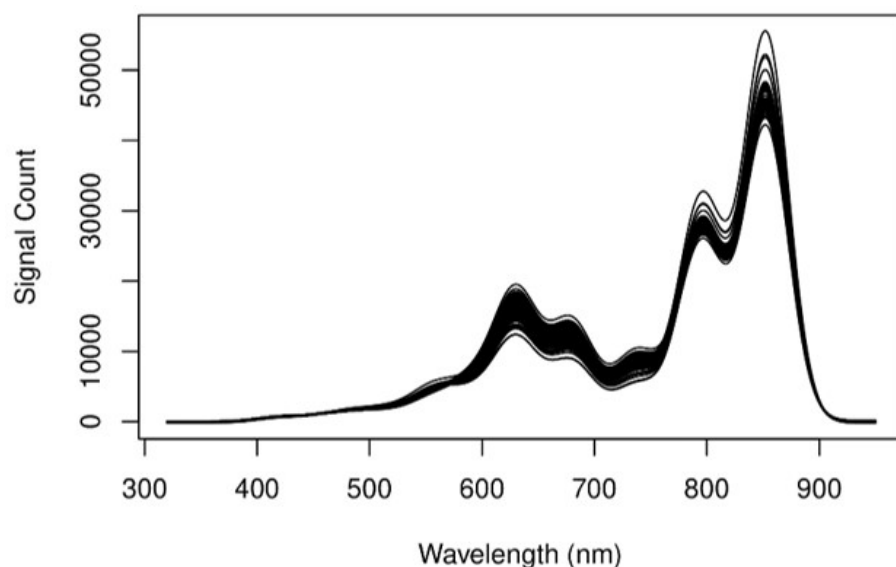


Figure 4.3: Blood Spectra

The figure 4.3 represents the hemogram spectra. The blood spectrum provides important insights into the composition and properties of the blood samples, particularly with respect to hemoglobin content and its oxygenation state, along with other factors. These features can be used in diagnostic or analytical contexts to assess the health or condition of the samples being analyzed. The primary signals in blood spectra come from components that strongly absorb light in the visible to near-infrared (Vis-NIR) spectrum, particularly hemoglobin (Hgb) and bilirubin (Bil). Red blood cells (RBCs) are the most prevalent cells, and hemoglobin largely determines the patterns of absorbance and transmittance in the spectra. Meanwhile, components present in

lower concentrations or with weaker absorbance/transmittance, such as bilirubin, manifest as interferences that affect the dominant spectral features, primarily those linked to hemoglobin. The variations in signal count across the spectrum could indicate differences in blood composition, such as varying levels of hemoglobin, oxygen saturation, or other blood constituents [38].

The Fig.4.4 shows the table of metric results for the PLS and SLAI methods.

Method	Metric	HGB (g/dL)	RBC ($10^6/\mu\text{L}$)	HTC (%)	WBC ($10^3/\mu\text{L}$)	PLT ($10^3/\mu\text{L}$)
PLS	R	0.5521	0.4272	0.5827	0.3079	0.2937
	SE	1.46	0.506	3.877	1.942	69.84
	MAPE (%)	11.02	11.81	7.93	30.19	35.04
	LV	3	3	3	4	2
SLAI	R	0.7496	0.6458	0.7603	0.6185	0.6507
	SE	0.9054	0.3293	2.431	1.279	43.31
	MAPE (%)	6.89	7.69	6.15	19.97	21.73
	LV	1	1	1	1	1
	Modes	20	20	20	30	30
	nPC	2	2	2	2	2

Figure 4.4: Table of metric results

4.1 Hemoglobin

4.1.1 Partial least squares

The PLS method shows a moderate positive correlation between predicted and actual hemoglobin values ($R \approx 0.552$). The standard error (SE) is relatively low ($SE \approx 1.46$ g/dL), suggesting moderate precision, though some variability remains. The mean absolute percentage error (MAPE) is about 11.0%, indicating acceptable but not highly accurate predictions. Three latent variables are used, indicating a moderately complex model.

4.1.2 Self learning artificial intelligence

The SLAI method demonstrates a stronger correlation ($R \approx 0.750$) between predicted and actual values compared to PLS, with a lower standard error ($SE \approx 0.905$ g/dL), indicating greater precision. The MAPE is also lower ($\approx 6.89\%$), suggesting better accuracy. SLAI uses only one latent variable, which indicates a simpler, yet more efficient model, with 20 modes and 2 principal components.

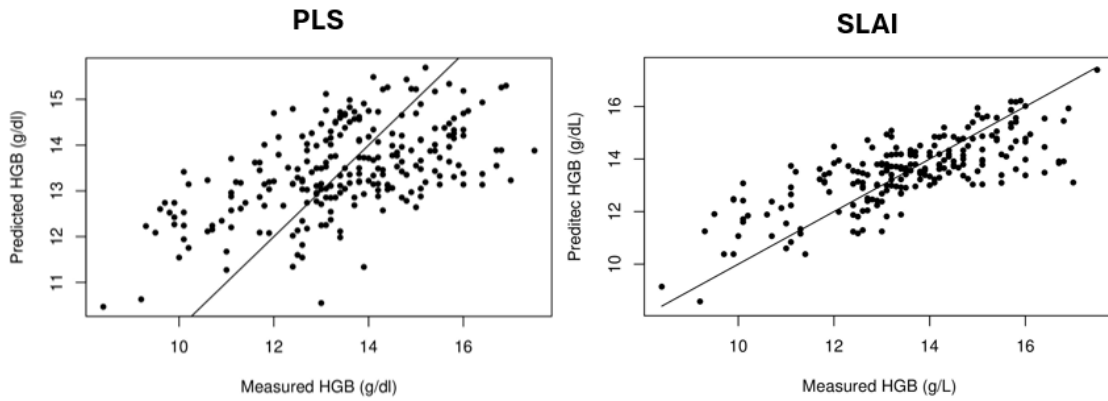


Figure 4.5: HGB - PLS and SLAI

The SLAI method outperforms PLS for hemoglobin, providing a stronger correlation, lower error rates, and better accuracy while maintaining a simpler model, as can be seen on Fig. 4.5.

4.2 Red blood cells

4.2.1 Partial least squares

For RBC counts, the PLS model shows a weak to moderate correlation ($R \approx 0.427$) and a relatively low SE, ($SE \approx 0.506 \times 10^6 \text{ cells}/\mu\text{L}$), the best SE within the PLS method, indicating moderate precision. The MAPE is approximately 11.8%, suggesting moderate accuracy. The model uses three latent variables, which adds moderate complexity.

4.2.2 Self learning artificial intelligence

The SLAI method achieves a higher correlation ($R \approx 0.646$), indicating better predictive capability, and has the lowest SE among all SE metrics ($SE \approx 0.329 \times 10^6 \text{ cells}/\mu\text{L}$), suggesting high precision. The MAPE is also lower at about 7.69%, indicating improved accuracy compared to PLS. SLAI uses one latent variable and 20 modes, similar to the hemoglobin model.

The SLAI method is clearly superior to PLS for RBC counts, providing a stronger correlation, higher precision, and more accurate predictions with a simpler model. The graphics that represent PLS and SLAI applied to RBC counts are shown below, Fig.4.6.

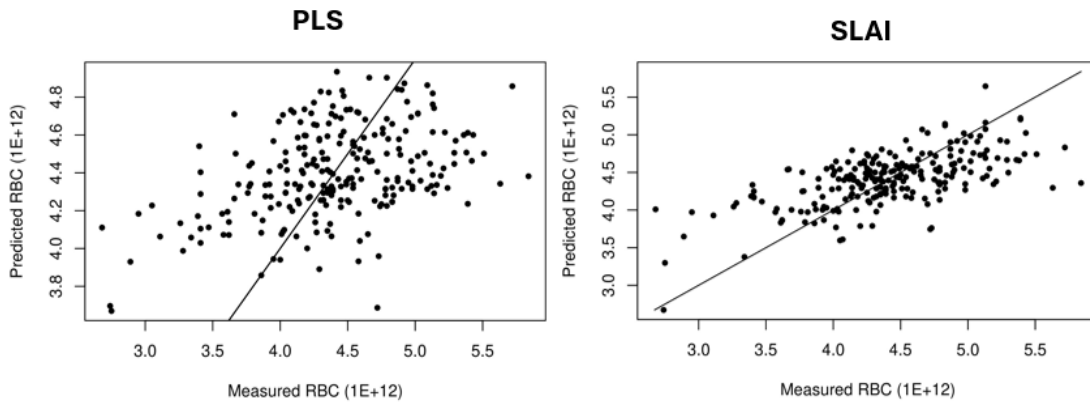


Figure 4.6: RBC - PLS and SLAI

4.3 Hematocrit

4.3.1 Partial least squares

For hematocrit, the PLS method shows a moderate correlation ($R \approx 0.583$), the best correlation within the PLS method, with a relatively high SE ($\approx 3.88\%$), indicating greater variability and less precision. However, the MAPE remains relatively low ($\approx 7.93\%$), the best MAPE within the PLS method, suggesting acceptable prediction accuracy. The model uses three latent variables, as seen with hemoglobin and RBC.

4.3.2 Self learning artificial intelligence

The SLAI method exhibits a much stronger correlation ($R \approx 0.760$), the highest among all correlation metrics, and a lower SE ($\approx 2.43\%$). It also has the lowest MAPE ($\approx 6.15\%$), indicating very accurate predictions for hematocrit. Only one latent variable is used, demonstrating model simplicity, along with two modes.

SLAI significantly outperforms PLS, providing a stronger correlation, lower error rates, and higher accuracy with a simpler model for predicting hematocrit. The results are shown in Fig.4.7.

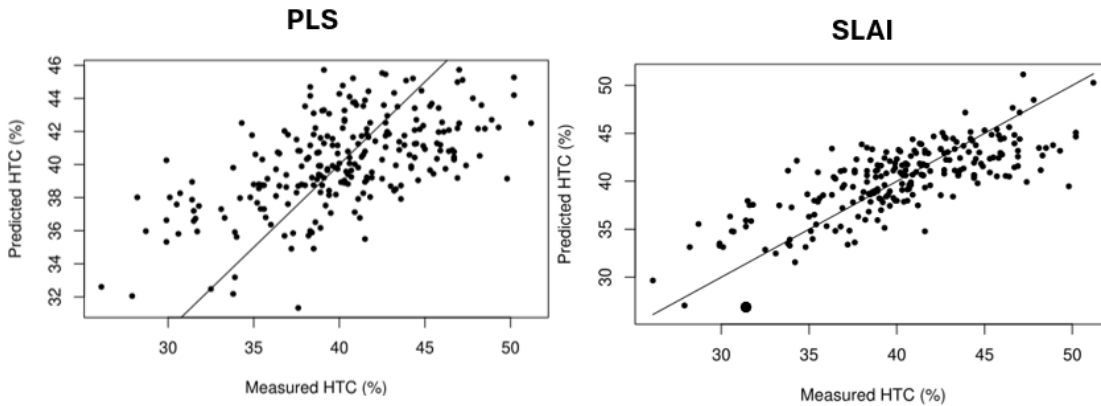


Figure 4.7: HTC - PLS and SLAI

4.4 White blood cells

4.4.1 Partial least squares

For WBC counts, the PLS model shows a low correlation ($R \approx 0.308$), indicating poor predictive performance and explaining only about 31% of the variance. The SE is relatively high ($SE \approx 1.94 \times 10^3$ cells/ μL), and the MAPE is also high ($\approx 30.2\%$), suggesting low accuracy. Four latent variables are used, the highest among all metrics, indicating a more complex model.

Due to the complex nature of the spectral data for WBC, the PLS model requires high dimensionality (4 LVs) to account for various interferences and achieve a reasonable correlation. However, because of many interfering spectral gradients, PLS struggles with detailed differentiation of covariance modes (CovM) in the data.

4.4.2 Self learning artificial intelligence

The SLAI method shows a marked improvement over PLS, with a moderate correlation ($R \approx 0.619$), lower SE ($SE \approx 1.28 \times 10^3$ cells/ μL), and a lower MAPE ($\approx 20.0\%$) compared to PLS, indicating better accuracy. It uses only one latent variable and 30 modes, making it a much simpler model.

While SLAI provides substantial improvement over PLS for WBC predictions, the overall prediction accuracy is still moderate, Fig.4.8.

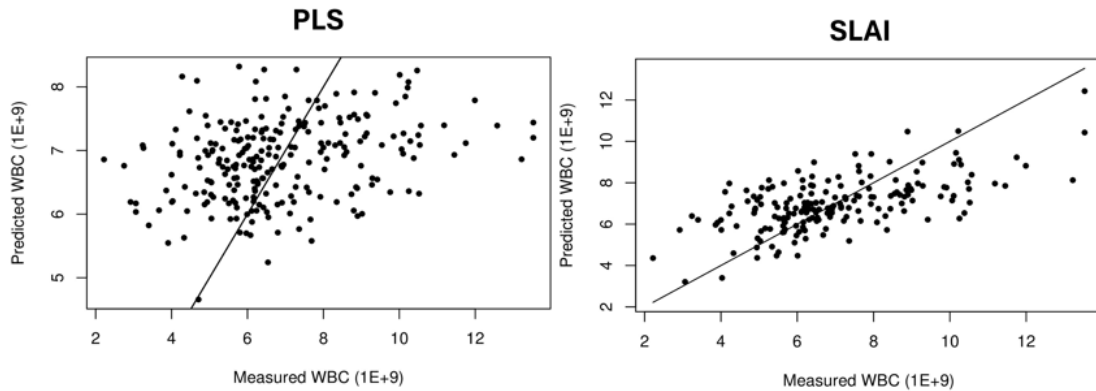


Figure 4.8: WBC - PLS and SLAI

4.5 Platelets

4.5.1 Partial least squares

For PLT counts, the PLS model has a very low correlation ($R \approx 0.294$), indicating poor performance. The SE is extremely high ($\approx 69.8 \times 10^6$ cells/ μL), suggesting very low precision, and the MAPE is also high ($\approx 35.0\%$), the highest among all metrics, reflecting poor accuracy. The model uses two latent variables, indicating moderate complexity.

PLS analysis indicates that the relationship between spectral data and PLT counts is unstable and nonlinear due to the low quantity of platelets and their interference with other major blood components. The model's performance is limited by its inability to handle scattered information across different interference modes.

4.5.2 Self learning artificial intelligence

The SLAI method shows a significant improvement over PLS, with a moderate correlation ($R \approx 0.650$), lower but still relatively high SE ($\approx 43.3 \times 10^6 \text{ cells}/\mu\text{L}$), and a reduced MAPE ($\approx 21.7\%$), indicating better accuracy but still with the highest error among the metrics.

SLAI improves predictions for PLT counts over PLS, but overall accuracy remains limited, reflecting the challenges of predicting platelets accurately with these models. The results are represented on Fig.4.9.

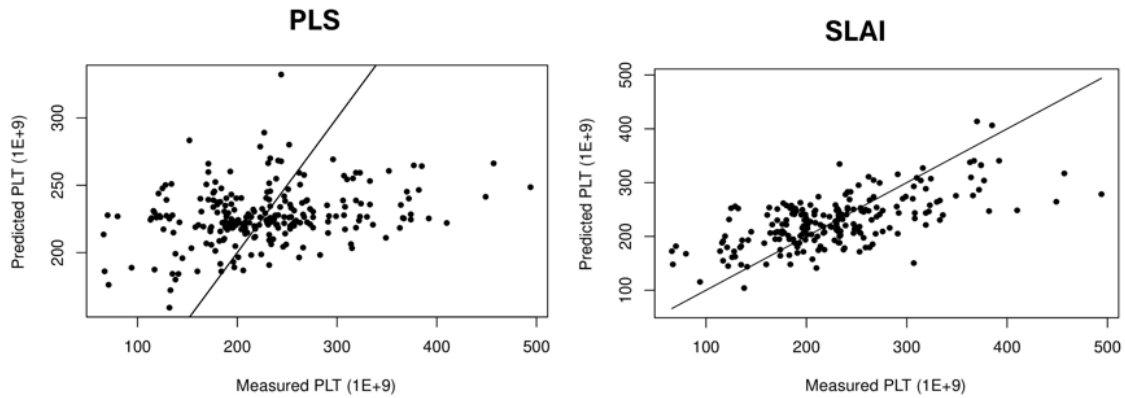


Figure 4.9: PLT - PLS and SLAI

For all blood metrics (HGB, RBC, HTC, WBC, PLT), the SLAI method consistently performs better than PLS in terms of correlation (R), precision (SE), and accuracy (MAPE). SLAI achieves correlations above 0.60 for all metrics, with the highest ($R \approx 0.760$) for HTC. This method also has the lowest SE, approximately ($0.329 \times 10^6 \text{ cells}/\mu\text{L}$) for RBC, and the lowest total error, MAPE of approximately 6.15%, for HTC. Red blood cells, haemoglobin (Hgb) and haematocrit (HTC) have strong absorption characteristics, which makes them more easily detectable by Vis-SWNIR.

SLAI achieves better results with a simpler model, using only one latent variable across all datasets, and optimized parameters (Modes and nPC), making it a more effective and efficient method for predicting blood metrics. The use of 2 principal components for all metrics indicates a consistent approach in balancing complexity and performance. The covariance matrix (CovM) captures the shared relationships among different blood components, showing a consistent interference pattern in the spectral data, where $t \approx u$.

HTC is the easiest metric to predict accurately with both methods, despite not having an excellent error standard. The prediction of WBC and PLT counts was more challenging due to their

complex relationships with other blood components and potential interferences. This outcome was anticipated based on the patterns observed in the correlation and PCA graphs presented earlier.

While WBC and PLT show strong correlation values comparable to those of the other variables using the SLAI method, and WBC has an acceptable standard error (SE) relative to the other variables, both WBC and PLT exhibit high prediction errors (MAPE) and PLT has a significantly high standard error in both methods (PLS and SLAI), indicating difficulties with the accuracy of the predictions for these variables.

White blood cells (WBC) are present in much lower proportions compared to red blood cells (RBC) in blood, making their detection in spectral data harder. The spectral information is primarily dominated by RBC and Bil, which can interfere with WBC. As a result, the contribution of WBC to the overall spectral variance is minimal, especially when compared to the overwhelming presence of RBC. Furthermore, WBC-related information is dispersed across the 200 to 800 nm range, often appearing as interferences within the spectral data predominantly influenced by hemoglobin [38, 90].

Quantifying platelets (PLT) accurately is particularly difficult due to several factors, such as their tendency to clump, which can result in erroneous counts and unreliable predictions. Platelets are found in much lower concentrations than red blood cells (RBCs), and their small size further complicates their detection using standard spectroscopy methods. Because of their minimal surface area and volume, platelets have limited capacity for light absorbance, leading to low spectral sensitivity and minimal contrast with hemoglobin. Moreover, there is significant spectral overlap between platelets and other major blood components, such as RBCs, HGB, and Bil, which further obscures their signals. The wide variability in platelet morphology, ranging from small, inactive cells to larger, activated forms with extensions or clots, adds further complexity [33]. It is also important to consider that, in stroke patients, platelets are more likely to form aggregates or be present in thrombi [91].

Furthermore, the presence of aggregated platelets can affect the accuracy of platelet and white blood cell counts, potentially leading to incorrect PLT results. Depending on the type of analyzer used, these aggregated platelets may be mistakenly counted as white blood cells or specifically as eosinophils [92].

These challenges can make it difficult to apply a straightforward linear model to platelets accurate detection and quantification. Despite these challenges, the SLAI method combined with Vis-SWNIR spectroscopy reduces the total error in PLT quantification to about 21.7%, a notable improvement over PLS.

Despite the limitations shown in this study, PLT quantification using Vis-SWNIR spectroscopy in conjunction with the new SLAI algorithm can attain a total error estimate of approximately 21.7%. Such result is below the total allowable error in humans defined by CLIA. For platelet counts, the CLIA standard allows a total variation of $\pm 25\%$. The SLAI method ($\text{MAPE} \approx 21.7\%$) falls within this acceptance range, indicating that the SLAI method could be considered acceptable for platelet counting according to CLIA standards. However, the PLS method ($\text{MAPE} \approx 35.0\%$)

exceeds this limit, suggesting that it is not suitable for clinical application based on current performance.

The table on Fig.4.10, represent the acceptance limits for proficiency testing by CLIA.

Hematology CLIA 2024	
Analyte or Test	NEW Criteria for AP
Cell identification	80% or greater consensus
White blood cell differential	TV $\pm$ 3 SD based on the % of different types of white blood cells in the samples
Erythrocyte count	TV $\pm$ 4%
Hematocrit	TV $\pm$ 4%
Hemoglobin	TV $\pm$ 4%
Leukocyte count	TV $\pm$ 10%
Platelet count	TV $\pm$ 25%
Fibrinogen	TV $\pm$ 20%
Partial thromboplastin time	TV $\pm$ 15%
Prothrombin time	TV $\pm$ 15%

Figure 4.10: 2024 CLIA Acceptance Limits for Proficiency Testing [93]

The CLIA regulations set quality standards for laboratory testing on human specimens, such as blood, body fluids, and tissues, to aid in the diagnosis, prevention, or treatment of diseases or for assessing health. The current performance of both the PLS and SLAI methods does not align with the CLIA acceptance criteria for RBC, HGB, HTC, and WBC counts, as all the MAPE values are above the permissible total variation limits. This indicates a need for further refinement to minimize errors and enhance accuracy if these methods are to be applied clinically with a POC device [94].

According to CLIA standards, the total acceptable variation for WBC counts is $\pm 10\%$. Both the PLS and SLAI methods exceed this limit, with MAPE values of approximately 30.2% and 20.0% respectively, showing that they do not fulfil the CLIA criteria for leukocyte counts. However, even with the Sysmex analyser, leucocyte counts can show errors of more than 10% due to cell interference, and microscopic counts, being qualitative, can show errors ranging from 20-30%. Therefore, in this context, the POC results for leucocyte levels can be considered qualitative.

Similarly, neither the PLS nor the SLAI method meets the CLIA criteria for RBC, HGB, and HTC counts, where the acceptable variation is $\pm 4\%$. This underscores the need for additional optimization to reduce error margins and enhance the accuracy of these measurements if they are intended for use in a clinical setting with a POC device.

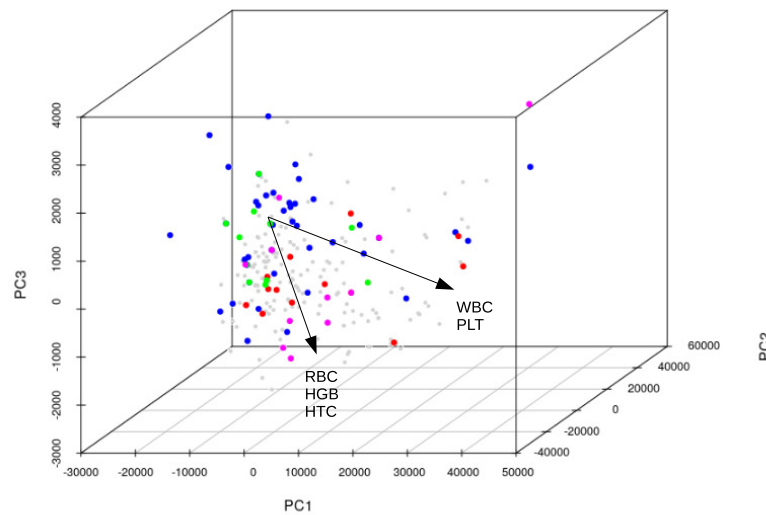


Figure 4.11: Spectra Space

Fig.4.11 represents the spectra space. The 3D PCA plot effectively visualizes the relationship between different blood components (RBC, HGB, HTC, WBC, PLT) and how they contribute to the variance in the dataset. HGB, RBC and HTC appear to be the primary contributors, reflecting their strong mutual correlation and their predominant influence on the overall data structure. PLT and WBC have a more significant influence on PC2, indicating a relationship between these variables. There is clearly a separation between the variances of these two groups of variables. A few points, particularly in the upper PC3 region, might be considered outliers.

Automated hematology has recognized limitations when conducting differential cell counts. Ideally, both WBC and PLT values should have been validated using blood smear microscopy counts. However, this method was beyond the scope of this master's thesis and should be employed in future research to build a knowledge base with the most accurate results possible.

Chapter 5

Conclusions

This chapter summarizes the main conclusions obtained from the developed work. Lastly, the steps to follow in future research are outlined.

This master's thesis addresses a critical need for rapid stroke diagnosis, especially in emergency medical situations where quick diagnosis is vital. Given that stroke is one of the leading causes of mortality and disability, this research has opened up the opportunity to significantly improve healthcare outcomes.

The project focused on evaluating and improving a POC spectral device capable of quantifying blood cells using advanced signal processing and artificial intelligence techniques. This POC technology, which has proven effective in veterinary applications, aims to deliver immediate results from just a drop of blood, making it portable, accessible, and efficient.

The analytical capacity of the POC spectral device for quantifying clinical blood parameters in human samples was analyzed. Spectral data were obtained from blood samples, and a hemogram count was performed, including hemoglobin, red blood cells, white blood cells, platelets and hematocrit count. A comparative analysis was conducted against results from the hospital's hemogram equipment (Sysmex), demonstrating that the Self Learning Artificial Intelligence method outperforms Partial Least Squares across all evaluation metrics, achieving higher correlation coefficients and lower total error rates. The Self Learning Artificial Intelligence algorithm combined with Vis-SWNIR spectroscopy achieves a total error of about 21.7% for platelet quantification, meeting the Clinical Laboratory Improvement Amendments standards for clinical use.

This study demonstrated that low-intensity, non-dominant, and multi-scale spectral interference can be effectively accessed by using the Covariance mode principle integrated within the Self Learning Artificial Intelligence method. Self Learning Artificial Intelligence can separate interference information based on the Covariance mode principle, enabling accurate quantification of blood cells. These results indicate that conventional methods, such as Partial least squares, may not be adequate for managing the complex interferences commonly found in blood samples.

The research also highlights the potential of Vis-SWNIR spectroscopy for non-invasive blood cell counting and demonstrates the feasibility of using Spectral POC technology as a reagent-free, portable hematology device. This technology could significantly improve healthcare accessibility by offering a cost-effective solution for frequent monitoring of chronic conditions, facilitating health assessments in resource-limited settings, and expediting responses to disease outbreaks. The integration of Internet of Things and Artificial Intelligence in the POC device allows real-time data analysis with minimal computational resources, marking a notable advancement in personalized medicine.

Furthermore, the thesis emphasizes the global impact of POC Health Technologies, demonstrating their relevance worldwide.

In conclusion, this research addresses a critical healthcare need by introducing innovative technological solutions for patient care, demonstrating the potential feasibility of using a low-cost, compact and reagent-free POC device to accurately quantify blood cells from a small sample. This technique could be an encouraging alternative to traditional methods, especially for POC applications. However, further research is essential to optimise the accuracy of the device and validate its performance in various clinical contexts.

5.1 Future work and perspectives

This project presents valuable opportunities for doctoral research, not only to address the limitations identified in this study but to further develop the aims of improving stroke diagnosis in hospital emergency rooms and reducing the time needed for diagnosis.

For future work, the following steps should be performed:

- Classification of the spectral signatures of stroke cases;
- Develop methods for classifying and quantifying platelet aggregates and thrombi by detecting changes in light scattering, using the POC device employing light spectroscopy to detect changes in scattering, regarding stroke predisposition;
- Enhance the use of advanced artificial intelligence systems, such as SLAI, and signal processing techniques to manage spectral interference and extract meaningful data;
- Conduct detailed investigations into thrombi in blood samples to determine their size, composition, and origin, which is a key long-term challenge;
- Use blood smear microscopy counts to determine inaccurate cell identifications and counts from automated hematology;
- Improve the POC optical sensitivity by manipulating the optical path length.

Future work should focus on increasing the sample size, refining AI algorithms, and exploring the application of the device to additional blood parameters and various clinical scenarios. To address the issue of platelet aggregates and thrombi, which complicate accurate counting of platelets and white blood cells, future research should incorporate blood smears to detect platelet clusters and ensure precise platelet counts. Regardless of the counting method, examining a blood smear for platelet clumps is essential before confirming the platelet result.

These efforts aim to facilitate rapid stroke diagnosis in emergency situations, ultimately reducing the time required to diagnose this critical condition.

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Scientific outputs

Posters in conference proceedings

2nd best poster presentation award at the International Congress of Sensors in Medicine 2024.

Soares ML, Barroso TG, Monteiro-Silva F, Queirós C, Santos F, Moura P, Macedo D, Oliveira M, Sousa MA, Antunes R, Furtado M, Gregório T, Martins RC (2024) Reagent-less Point-of-Care for Hemogram Analysis in the Emergency Room using a drop of blood: An Assessment Study, *International Congress of Sensors in Medicine 2024*, 5-7 March, London.