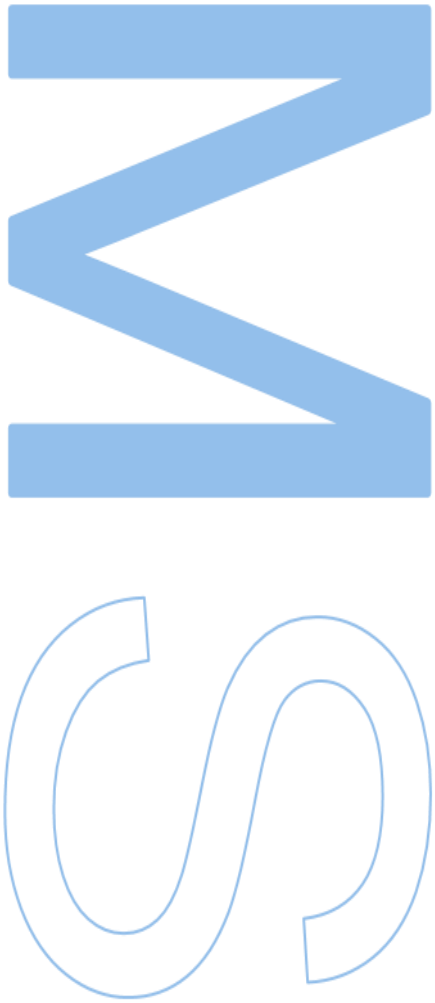
Medical Tricorder- Spectroscopy Point-of-Care Photonics Technology



Luís de Jesus Manuel Monteiro

MSc. Medical Physics

Department of Physics and Astronomy

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Supervisor
Rui da Costa Martins, Senior Researcher

Applied Photonics Research Center – INESC TEC


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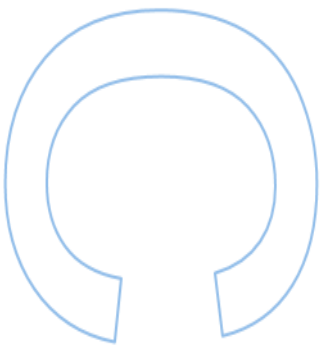

Dedicated to

My wife Helena Monteiro, and my mother Carolina Manuel Bota for all your confidence in my potential.

Sworn Statement

I, Luis de Jesus Manuel Monteiro, enrolled in the Master's Degree in Medical Physics at the Faculty of Sciences of the University of Porto hereby declare, in accordance with the provisions of paragraph a) of Article 14 of the Code of Ethical Conduct of the University of Porto, that the content of this dissertation reflects perspectives, research work and my interpretations at the time of its submission.

By submitting this dissertation, I also declare that it contains the results of my research work and contributions that have not been previously submitted to this or any other institution.

I further declare that all references to other authors fully comply with the rules of attribution and are referenced in the text by citation and identified in the bibliographic references section. This dissertation does not include any content whose reproduction is protected by copyright laws.

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Luís de Jesus Manuel Monteiro

Setembro/2022

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Firstly, I would like to thank God for the grace of life.

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Universidade do Porto

Resumo

Faculdades de ciências da universidade do Porto

Departamento de Física e Astronomia

MSc. Medical Physics

Medical Tricorder, Spectroscopy Point of Care Photonic Technology

By Luís de Jesus Manuel Monteiro

Antecedentes: A Tecnologia Fotônica de Espectroscopia PoC é um dos poderosos sistemas de diagnóstico, é uma técnica que pode testar resultados rapidamente permitindo imediata assistência aos pacientes. **Objetivo**: Tive como objetivo fazer uso da poderosa capacidade analítica da Tecnologia Espectral PoC para quantificação de parâmetros e classificação de doenças por vias de análise clínica do sangue. **Métodos**: Os métodos atuais de quimiometria e inteligência artificial são imprecisos para lidar com a interferência multe escala complexa de constituintes do sangue em tecnologias de ponto de atendimento (PoC) de espectroscopia do visível de ondas curtas próximo ao infravermelho (Vis-SWNIR). Esta pesquisa apresenta um novo método de inteligência artificial de autoaprendizagem para processamento espectral baseado na busca de modos de covariância com correspondência direta com a lei de Beer-Lambert (BLL) para solucionar as limitações do estado da arte. Os resultados das amostras de sangue de cães e gatos saudáveis e doentes foram estudados para comparabilidade entre a quimiometria de última geração: modelo linear, similaridade, mínimos quadrados parciais, mínimos quadrados parciais locais e redes neurais artificiais usando o analisador Mindray BC 5000 Vet com a inteligência artificial de autoaprendizagem (SLAI) usando o dispositivo PoC. **Resultados**: os resultados apresentados com o dispositivo PoC melhora o estado da arte, mas fica longe do apresentado pelo ASVCP que está no intervalo de 7% a 50% do TEa, e o dispositivo PoC apresenta entre 13% a 60 % de TEobs. É útil para garantir que a pesquisa mantém seu nível de relevância.

PALAVRAS-CHAVE: SLAI, acurácia, hemograma, gato, cão, espectroscopia, PoC. TEa, TEobs.

Porto University

Abstract

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Medical Tricorder, Spectroscopy Point of Care Photonic Technology

By Luís de Jesus Manuel Monteiro

Background: Spectroscopy PoC Photonic Technology is one of the powerful diagnostic systems, it is a technique that can rapidly test results allowing assisted immediate patients. **Objective**: I aimed to Make use of the powerful analytical capacities of the Point-of-Care Spectral Technology for blood clinical analysis parameter quantifications and disease classification. **Methods**: Current chemometrics and artificial intelligence methods are imprecise to deal with complex multi-scale interference of blood constituents in visible shortwave near-infrared (Vis-SWNIR) spectroscopy point-of-care (PoC) technologies. This research presents a new self-learning artificial intelligence method for spectral processing based on the search of covariance modes with direct correspondence to the Beer-Lambert law (BLL) to solve the limitation of the state-of-the-art. Blood sample results of healthy and ill dogs and cats were studied for comparability between state-of-the-art chemometrics: linear model, similarity, partial least squares, local partial least squares, and artificial neural networks using the Mindray BC 5000 Vet analyser with the Self Learning-Artificial Intelligence (SLAI) using PoC device. **Results**: the result presented with the PoC device improve the state-of-the-art but it is far from that presented by ASVCP which is an interval of 7% to 50% of TEa, and the PoC device presents between 13% to 60% of TEobs. It is useful to assure the research maintains its level of relevance.

KEYWORDS: SLAI, accuracy, blood count, cat, dog, Spectroscopy, PoC. TEa, TEobs

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List of Abbreviations

ANNPCA	ARTIFICIAL NEURAL NETWORKS PRINCIPAL COMPONENT ANALYSIS
ANNPLS	ARTIFICIAL NEURAL NETWORKS PARTIAL LEAST SQUARES
AI	ARTIFICIAL INTELLIGENCE
ANN	ARTIFICIAL NEURAL NETWORKS
ASVCP	AMERICAN SOCIETY FOR VETERINARIAN CLINIC PATHOLOGY
BAS	BASOPHILS
Bil	BILIRUBIN
BLL	BEER-LAMBERT LAW
CCD	CHARGE-COUPLED DEVICE
CBC	COMPLETE BLOOD COUNT
CHVP	CENTRO HOSPITALAR VETERINÁRIO DO PORTO
COVM	COVARIANCE MODE
DBG	DYNAMICAL BIOMARKERS GROUP
DNA	DEOXYRIBONUCLEIC ACID
EOS	EOSINOPHILS
FCUP	FACULTY OF SCIENCES OF THE UNIVERSITY OF PORTO
HE-NE	HELIUM-NEON
HGB	HEMOGLOBIN
HTC	HEMATOCRIT
ICSH	THE INTERNATIONAL COUNCIL FOR STANDARDIZATION IN HEMATOLOGY
IOT	INTERNET OF THINGS
INR	INTERNATIONAL NORMALIZED RATIO (COAGULATION)
LED	LIGHT-EMITTING DIODE

LYMPH	LYMPHOCYTES
LV	LATENT VARIABLES
LOCPLS	LOCAL PARTIAL LEAST SQUARES
MASEP	MEAN ABSOLUTE STANDARD ERROR PERCENTAGE
MIMED	MAGNETIC INTEGRATED MICROFLUIDIC ELECTROCHEMICAL DETECTOR
MEQ-LAMP	MICROFLUIDIC ELECTROCHEMICAL QUANTITATIVE-LOOP AMPLIFICATION
MCV	MEAN CORPUSCULAR VOLUME
MCHC	MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION
MONO	MONOCYTES
MRNA	MESSENGER RIBONUCLEIC ACID
MPV	MEAN PLATELET VOLUME
NEU	NEUTROPHILS
NIH	NATIONAL INSTITUTES OF HEALTH
NIBIBA	AMERICAN NATIONAL INSTITUTE OF BIOMEDICAL IMAGING AND BIOENGINEERING
PC	PRINCIPAL COMPONENT
PCA	PRINCIPAL COMPONENT ANALYSIS
PCT	PLATELETCRIT
PDW	PLATELET DISTRIBUTION WIDTH
PLS	PARTIAL LEAST SQUARES
PLT	PLATELET
POC	POINT OF CARE
R	PEARSON CORRELATION COEFFICIENT
RBC	RED BLOOD CELL

RDW-CV	RED CELL DISTRIBUTION WIDTH - COEFFICIENT OF VARIATION
RDW-SD	RED CELL DISTRIBUTION WIDTH – STANDARD DEVIATION
RI	REFERENCE INTERVAL
ROI	REGIONS OF INTEREST
SLAI	SELF LEARNING-ARTIFICIAL INTELLIGENCE
SWCNTs	SINGLE-WALLED CARBON NANOTUBES
TEA	ALLOWABLE TOTAL ERROR
TEOBS	OBSERVABLE TOTAL ERROR
UP	UNIVERSITY OF PORTO
UV- VIS-SWNI	ULTRAVIOLET-VISIBLE NEAR-INFRARED
VIS-SWNI	VISIBLE SHORTWAVE NEAR-INFRARED
WBC	WHITE BOOD CELL

FRAMEWORK

The theme of my research project is **Medical Tricorder-Spectroscopy Point-of-Care Photonics Technology**. In order to prevent diseases and to assure general health, we need to follow many procedures, and clinical analysis is one of them, so I will present here a deeper understanding of new ways to determine the body's predisposition to develop certain diseases and if the organism is healthy or not, with the help of the Medical Tricorder based on spectroscopy.

The research is divided into three parts: in the first one, I am going to present closely, the real motivation, relevance, difficulties, state-of-the-art, and objectives, so that I can introduce my strong lines that will guide this dissertation; in the second part, the material and methods that will guide me to reach my goals will be presented and in the last part the results will be discussed to disclose a better conclusion of the thesis.

Before developing anything about the technology, I think that is imperative to understand the concept of this point-of-care (PoC) technology, which is the diagnostic platform that evaluates the biomarkers that we need for an underlying biochemical or physical characteristic of an individual situation. It is thoroughly important to know that, these biomarkers diagnose and determine the real risk and the severity of the illness, and the individual response to the treatment [1].

I.1- Relevance

In this thesis, the use of Spectroscopy PoC Photonic Technology in the healthcare sector was demonstrated, showing the potential to determine several hematological and biochemical parameters in liquid clinical samples, such as - urine, saliva, sweat, and blood.

Spectral PoC photonic technology can be able to perform the right predictions with little information is paramount. It translates into low consumption of computing resources, enabling the development of the internet of things (IoT), with embedded artificial intelligence (AI). Biochemical and physical variability is very significant, and it is not always possible to cover all the information that relates spectral interference to composition in a complex matrix such as blood [2].

My real line to develop this theme is to present the capacity of this technology to make the clinical blood analysis using Spectroscopy PoC which is a recent research area with great potential in healthcare by enabling the possibility of making clinical blood

analysis reagent-less and in the clinical setting (anywhere and anytime), making this device a powerful asset. This description can also be applied to a new generation of sensing and imaging devices. Recent miniaturisation of this kind of technology, the advent of smartphones with cameras and high quality in data-transmission capabilities, and other factors have led to the development of a host of instruments that might be described as tricorders, or at least as tricorder-like [3]. Its ability to determine lesions earlier on the strength of information that we can take from the spectrum of light that is transmitted from the blood makes it easy for us to look for some mechanism to identify and protect against any kind of disease. Its lower cost and other potentials that I have already presented here, make this device a powerful element of healthcare in all countries of the world independently of their economic level. An equally important factor is the simplicity to work with the tricorder because it is not necessary to have deep training (each one can work with it) [4]. The above-mentioned reasons motivated me to do this research.

I.1.1- Scientific Challenge in the Use of Spectroscopy

- Transform this technology into one present appliance in our daily life, so that we can monitor our health state;
- Understand how the information is linked and organised with the cellular quantification in the hemograms, for example, to be able to identify the difference between normal and anemic blood;
- The inevitable use of processing methods conjugated with AI to join the information;
- The overlapping spectral bands (multi-scale interference), of many different constituents, which focused on concentration and molar extinction coefficients outcoming the complete difference of the interference between intensities [2];
- The absorbance bands of pure constituents are caused by typical sample properties, which depend on the molecular state due to PH, avoidable or reversible reactions with another analyte, or even scattering characteristics owing to particle size [2].

I.2. Difficulties

In every work, difficulties reaching the objectives are always faced therefore, let me enumerate one by one every difficulty that I have had in the course of the work:

- Difficulty to find specific bibliography;
- COVID-19 made it impossible to start the study in the predicted period;
- The widespread application of this technology is still not possible, despite its potential impact on healthcare and patient outcomes, we can faced also difficulties in other similar research [5];
- The difficulty in obtaining a more affordable and autonomous tricorder capable of giving us global healthcare with help of the spectroscopy PoC photonic technology, once developing the PoC technologies, must require a continuous evolution of innovation in tricorder, information, and communication technologies [6].

I.3- **Important Physics Concepts Used in the Development of Spectroscopy PoC Photonic Technology**

To understand the function of the optical device is important to know how light and matter interact. I will shortly present here the basic principles behind this technology.

Today, laser and LED light are common tools for medical examination and treatment. Consequently, there is a medical interest in the light-scattering properties of tissue for diagnostic purposes and optical analysis of blood for blood-related diseases [7].

Reflection and refraction of electromagnetic radiation are widely used in various fields of science and technology to study the structure and properties of different elements.

In **reflection**, when a beam of light strikes an interface, some light is always scattered backward [8, 9]. In general, a reflecting surface is a physical boundary between two materials of different refraction indexes such as air and tissue. The simple law of reflection requires that the wave of the incident, reflected beams and the reflecting surface lie within one plane called the plane of incidence. It also states that the reflection angle θ´ equals the angle of incidence θ as shown in Fig. 1 [10].

$$\theta = \theta' \qquad (1)$$

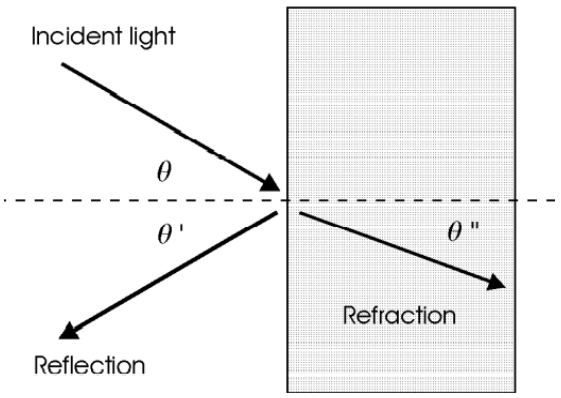


Figure 1. The geometry of reflection and refraction in a smooth surface [10].

In **refraction** when a plane wave falls onto a boundary between two homogeneous media of different optical properties, it is split into two waves, and the transmitted wave proceeds into the second medium [8, 9, 11]. Refraction always comes together with reflection [12].

The mathematical expression of Snell's law given by:

$$\frac{sin\theta}{sin\theta'} = \frac{v}{v'} \qquad (2)$$

$$n = \frac{c}{v} \qquad (3)$$

$$n' = \frac{c}{v'} \qquad (4)$$

Where: θ => is the angle of incidence; θ' => is the angle of reflection; θ'' => is the angle of refraction; v and v' => are the speed of incident and reflected light; n and n' are the corresponding refraction indexes and c is the speed of light in vacuum.

Hence, Snell´s law may turn into:

$$nsin\theta = n'sin\theta'$$

It is essential to know that: the reflection and refraction of the waves on surfaces of real bodies (particularly in biological tissues) are accompanied by effects that are not observed on smooth surfaces [13].

Absorption is the process by which electromagnetic waves transfer energy to matter [14]. During absorption, the intensity of incident light is attenuated in passing through a medium. A substance is said to show general absorption if it reduces the intensity of all wavelengths in the considered spectrum by a similar fraction [10].

The ability of a medium to absorb radiation depends on several factors, mainly the electronic constitution of its atoms, the wavelength of radiation, the thickness of the absorbing layer, and internal yardsticks such as the temperature or concentration of absorbing agents [10]. The law that describes the effect of thickness or concentration on absorption is the Beer-Lambert law.

Mathematical expression of Beer-Lambert law:

$$I(z) = I_0 e^{-\alpha z} \qquad (5)$$

And

$$I(z) = I_0 e^{-k'cz} \qquad (6)$$

Where: $I(z)$ and I_0 are the intensities at a distance z and incident intensity, α is the absorption coefficient of the medium, z is the optical axis, k´ depends on internal parameters other than concentration and c denotes concentration of absorbing agents.

One of the most important intrinsic properties of the cells and tissues is light absorption which can be harnessed in diagnostic applications [15]:

The ability of electromagnetic radiation to penetrate tissue, interrogate the tissue constituents, then escape the tissue for detection is key to diagnostic applications [16].

Light absorption can be analysed by various physical (electronic) and chemical (molecular) methods [13].

Scattering processes involve the simultaneous (instantaneous) absorption of an incident photon and the emission of another photon [17]. Still, Scattering takes place when the frequencies of light do not correspond to the natural frequencies of particles [10].

Light scattering is capable to provide structural and functional information about the tissue [18].

The scattering is divided into two main groups: elastic and inelastic scatterings.

In ***elastic scattering***, the scattered radiation is of the same energy as that of the incident radiation, and the total field measured will be the sum of the incident and scattered fields, always conserving both energy and momentum as long as the absorbed energy is taken into account [10] [15, 17, 19, 20].

Elastic scattering can be classified into three main groups in terms of the scatterers' relative size to the wavelength: (i) Rayleigh scattering, where the wavelength is much smaller than the scatterers; (ii) Mie scattering, where the size of the scatterer is comparable to the wavelength; and (iii) geometric scattering, where the scatterer is much smaller than the wavelength [15].

Inelastic scattering is denoted when light scattering originates from the interaction of photons with structural heterogeneities present inside material bodies at the wavelength scale and if the frequency of the scattered photon is lower or higher than the incident photon [15] [10, 17, 21, 22].

Inelastic light scattering or Raman scattering is the scattering of electromagnetic radiation by a medium, wherein the scattering process excitations are created (Stokes process) or annihilated (Anti stokes process) within the medium [21].

Raman scattering was a phenomenon that was discovered long before the necessary technology was available for routine analysis of soft matters such as food. Consequently, Raman spectroscopy has been regarded as an exotic technique with no broad utility [23]. Nevertheless, the scattering of radiation in molecules is undoubtedly one of the most effective methods of investigating the kinetics of various fluctuations and intermolecular interactions and has been repeatedly used with success for these purposes [24].

I.4- Technological Principles Used in PoC Technology

To analyse and interpret the data, in PoC technology there are different principles to make this technology one reality:

1- Flow cytometry can measure the particles such as microorganisms, nuclei, and chromosome preparations in the fluid stream or optical and fluorescence characteristics of the single when they pass through a light source [25].

The basic functional principle used in this kind of technology is that toward cells are focused by a laser beam of light that passes through each cell individually. Information about scatters of radiation as well as the loss of electromagnetic radiation owing to cellular absorption is recorded at many deviating angles from the original laser beam angle. The light detector captures the light reflected in the cells. Some data that are collected are cell size, cell shape, nuclear shape, and cytoplasmic complexity. Yet, more than 4 dimensions of information are considered. The end of the process is a

massive accumulation of individual information of data for each cell to assist with cellular identification. This principle has the most significant advantages when it comes to white blood cell (WBC) differentiation [26];

2- Electrical impedance or the Coulter principle, can count and size cells, based on the measuring changes in electrical resistance produced by nonconductive blood cells suspended in an electrolyte solution. This technique is used to determine essentially the number and volume of erythrocytes and platelets.

In the working principle, the blood is diluted in an isotonic solution inside the device, and a small opening between electrodes is a sensing zone through which suspended cells pass one by one through an electric voltage field where they induce a pulse according to their size; changes become an accurate measure of the real number of cells present, as well as to determine its volume [27];

3- The laser light scattering principle consists of nonflowing laser light scatter for automatically counting and classifying the blood cell; it is possible to calculate the size of the particle by taking into account the diffraction pattern because, when the laser beam passes through a sample of scattered particles if the particle is large the scatter light will produce the small-angle concerning to laser beam (the inverse occurs when the particle size is small).

The narrow laser radiation pass-through for a light-expanding lens, a pinhole filter, and a lens collimated lens were altered into uniform wide parallel radiation focusing on a blood sample contained in the sampling pool and was scattered by the blood sample. The scattered radiation is collected by a linear charge-coupled device (CCD), which is positioned on the back focal plane of a Fourier lens and converts the light into an electrical signal by the CCD. This signal is converted into a digital signal and collected by the data acquisition system. Where the digital signal is transmitted to a computer for an interface circuit and is processed by the computer and the result can be obtained. It is important to highlight that this laser is normally a helium-neon (He-Ne) laser with 632,8 nm of wavelength and 5 mW of power [28].

In the table below, I summarised the basic principles used by PoC technologies and our technology.

Table 1. Technologies Types of the PoC

Different Technologies	Working Principle	Advantages	Disadvantages	Reference
Spectroscopy PoC Photonic Technology	LED emits light in the capsule with an opposing mirror containing the sample, and a spectrometer to capture the spectrum of the light from the sample	rapid test results, reagent-less, no calibration worries, high results accuracy of the hematologic and biochemical test, analysis of different body fluids, easy to miniaturise, use SLAI, integration in the smartphone.	Carryover mistakes of the calibration machine (our results should not be better than the calibration machine).	[2, 29, 30]
Flow Cytometry	Toward cells is focused on a laser beam of light that passes through each cell individually. All Information owing to cellular absorption is recorded at many deviating angles from the original laser beam angle	measure microorganisms, nuclei, and chromosome; determine WBC differentiation; measures cell size and complexity.	Reagent, calibration preoccupation, large sampling, fluorescence characteristics of the single cell,	[25] [26]
Electrical Impedance	The blood is diluted inside the device, and with a small opening between electrodes, the cells pass one by one through an electric voltage field where they induce a pulse according to their size; changes	Count and size cells determine essentially the RBC and platelets	Difficult to miniaturization, Reagent, calibration preoccupation; large sampling; include a great deal of analyser	[27]

	become an accurate measure of the real number of cells and volume.		maintenance and cleaning.	
Laser Light Scattering	Narrow laser radiation focused on a blood sample is scattered and the scattered radiation is collected by a linear CCD, which is positioned on the back focal plane of a Fourier lens and converts the light into an electrical signal the CCD. This signal is converted into a digital signal and collected by the data acquisition system	laser light scatter for automatically counting and classifying the blood cell	Reagent, calibration preoccupation	[28]

I.5- State-of-the-Art

The original 1960s Star Trek series took place in a universe of the future with personal communicators [31], which take profit of particular significance, given that consumer electronic technologies can no longer be seen only as a complementary element besides professional medical procedures but are increasingly able to provide medical diagnoses and monitor diseases without the help of medical experts. This implies that low-cost consumer electronic technologies empower consumers to better monitor their health and promote individual self-care shortly [32].

After the year 1960, the issue of tricorder has been fixed only like a fiction situation, nevertheless, the period of 2012-2017 marked the boost of the investigation that transformed the fiction situation into person-centric solutions, with the presentation of the Qualcomm Tricorder XPRIZE, was a global competition to stimulate innovation and integration of advanced technologies, enabling reliable health diagnoses anywhere and anytime. The competition called for the development of a device that could diagnose

12 diseases (and the absence of disease) and capture 5 real-time health vital signs independently from a healthcare professional or facility [33].

The technologies for medical conditions in the consumer electronics category must fulfil three criteria: these technologies are proven to be precise in measurement, simple to use by private consumers, and available for private purposes.

Technologies that had been presented by some finalists of the XPRIZE challenge were:

- Intelesense-Scanadu: Intelesens Ltd, a medical technology company that specializes in intelligent wireless vital signs monitoring, and Scanadu Inc. The ultimate goal was to design a tricorder device for vital sign monitoring capable of changing the healthcare industry by putting medical devices back into the hands of individuals [32];
- Danvantri: dedicated to developing affordable but high-quality healthcare solutions based on mobile technologies. The main focus lies on the prevention of diseases by early detection made possible with an all-in-one handheld device [32];
- DMI: This device received funding from NASA, the Gates Foundation, and the National Institutes of Health (NIH). A single drop of blood is taken by the new rHEALTH X1 prototype, which applies it to nanoscale test strips, analyses the sample and links it with possible diagnoses [32];
- Dynamical Biomarkers Group (DBG): The main goal was to design light-weight and portable diagnostic instruments with high diagnostic precision and offer a good user experience and a flexible exchange between medical data and the cloud [32];
- Cloud DX: The company worked towards a medical device that allows ordinary people to monitor their health status, diagnose selected diseases and get a sense of when they should seek professional medical treatment [32];
- Final Frontier Medical Devices: announced as the highest performing team, this prototype was a tricorder called DxtER. This tricorder is an autonomous medical diagnostic device that leverages technological advances in wireless monitoring, artificial intelligence, and affordable PoC biomedical processes. Comprises several innovative sensors that together form a comprehensive healthcare kit, which collects and interprets data to diagnose selected medical conditions in real-time and recommend appropriate actions [32, 33].

In 2007 Bode *et al*. have guaranteed that the measurement of hemoglobin A1C has long been accepted as the major indicator of glucose control over time, based on charge difference structure. These technologies are frequently employed in expensive laboratory instruments. The recent A1C technology has been incorporated into PoC devices, enabling rapid availability of A1C measurement, and greatly facilitating diabetes care in general practice. They have shown the result in 5 minutes. Enhanced product performance allows the A1CNow+ to report a precise answer in 3 minutes [34].

In 2017 Khan *et al*. presented new advanced techniques incorporated into devices allowing the early detection and diagnosis of many oral and systemic diseases in a non-invasive, easily monitored, and less time-consuming way. Liquid biopsy, electric field-induced release, and measurement, biosensors, smartphone technology, and microfluidics, are the latest technologies for detection systems and clinic utilities. They have been based on a futuristic perspective of saliva diagnosis, reducing hospital stays by replacing screenings. The well-known examples of PoC Technology for self-monitoring of a blood glucose meter, coagulation (INR), and pregnancy test kits using urine samples have become over-the-counter products to be sold. Measuring the molecular level biomarkers in the form of proteins, mRNA, and DNA, they have used electrolytes and small molecules by the microfabrication technique. Current emerging technologies provide new perspectives on PoC diagnostics in a variety of lab-on-chip techniques, showing the possibility to detect and diagnose multiple diseases simultaneously with the help of biomarkers [1].

Abbasi *et al*. (2019) demonstrated the propriety of the lab-on-chip/µ technology platform for a regulatory grade mobile instrument for CBC hematology test, for rapid diagnostics at the PoC in resource-poor settings, their goal leveraging advances in microfluidics and, lab-on-chip fabrication techniques to miniaturise the conventional cytometer and bring down the cost significantly. This device may be able to operate autonomously without skilled manpower. It is important to highlight that the diffusion across even a small distance in a microfluidic channel could take several tens of minutes and the sample preparation sequence of RBC and thrombocytes is different than in the preparation of the sample for WBC [35].

Thongsahuan *et al*. (2020) presented the Mindray BC-5000 vet hematology analyser which generates the complete blood count (CBC). This device proved to be a suitable instrument for routine analysis of canines and felines with various hematological abnormalities; the software was developed for the analysis of blood from 13 animal species, providing 23 parameters including the 3 parts of the CBC analyser. Mindray BC-5000 Vet has been considered a last-generation solution for vet clinics, using several

working principles of the PoC technologies: Flow Cytometry combined with Tri-Angle Laser Scatter and Chemical Dye for WBC Differential count; Electrical Impedance principle for RBC and PLT counts; Colorimetric Method Cyanide-free for Hemoglobin determination [36].

Kim-Lina *et al*. (2022) presented a novel laser and impedance-based PoC hematology analyser, the vCell 5 (Scil Animal Care), supplying a CBC with leukocyte differential count, recently introduced to veterinary laboratories. They evaluated the analyser for use in canine and feline enclosing method comparison and assessment linearity, precision, and carryover. In increasing the widely established impedance principle for erythrocyte and platelet counts this PoC uses laser light flow cytometry for 5 parts leukocyte count. The result is displayed numerically and graphically as leukocyte scattergrams, and erythrocyte and platelet histograms, within 2 minutes [37].

It is important to underline the unique papers that in the state-of-the-art present a different technique and that do not join together different working principles to achieve and determine several yardsticks in a blood test.

In 2019, Martins *et al*. presented their Biophotonic device for the PoC, real-time, and non-invasive determination of different parameters that may be of diagnostic relevance. This optical device allows the characterisation of the elements that are in body fluids or tissues, using LED as a source for emitting light onto the sample, and a spectrometer to capture the spectrum of the light from the sample (light from the sample being of transmittance, reflectance, or Raman scattering of the emitted light). The data processing module is configured to change the recorded spectrum by conversion matrix into a standardized spectrum, so, the conversion matrix is obtained by calibrating of the optical system spectrum response against spectrum reference, and pre-processed spectrum with pre-obtained spectrum bands for each relevant parameter (the spectrum being contained within ultraviolet-visible near-infrared) UV-Vis-NIR wavelengths [29].

Martins *et al*. (2021) have shown that spectroscopy is one of the major powerful technologies for PoC miniaturisation owing to its capacity to analyse lower sample quantities and results in real-time. They presented here a feasibility study for the direct detection of WBC counts in dog blood by Vis-NIR spectroscopy for veterinary applications, applying here self-learning artificial intelligence as a new advanced method for high-precision in the quantification of the spectrum information [30].

Martins *et al*. (2021), in their thorough investigation of PoC Vis-SWNIR spectroscopy toward reagent-less technology, have presented that the relevance of the

research consists in the fact that they were able to improve current optical PoC technologies that are affected by spectral interference and displacing onto micro-sampling and reagent-less technologies in veterinary medicine and healthcare diagnostics. They have shown a new self-learning artificial intelligence method for spectral processing on the search of covariance modes with a direct link to the Beer-Lambert Law (BLL). This method outperforms the state of the art, providing a high analytical quality quantification according to the veterinary pathology guidelines, whereas common methods cannot [2].

Besides the technologies that I present here, we have many others that are in research, so, it is not my goal to develop them, I present them in a summarised table.

Table 2- Technologies Types of the PoC in research

Different Technologies	Working Principle	Advantages	Disadvantage	Reference
Spectroscopy PoC Photonic Technology	LED emits light in the capsule with an opposing mirror containing the sample, and a spectrometer to capture the spectrum of the light from the sample	rapid test results, reagent-less, no calibration worries, high results accuracy of the hematologic and biochemical test, analysis of different body fluids, easy to miniaturise, use SLAI, integration in the smartphone.	Carryover mistakes of the calibration machine (our results should not be better than the calibration machine).	[2, 29, 30]
Lab-on-chip	Microfluidic calorimetric, Magnetic integrated microfluidic electrochemical detector (MIMED), Microfluidic electrochemical	a chip can fabricate numerous microchannels, multiple testes in a single cartridge, multiple reactions can be analysed from a single drop of blood, low cost	Delay presenting results (5 min to 3,5 h), can limit device sensitivity (low temperature and clog).	[38-43]

	quantitative-loop mediated isothermal amplification (MEQ-LAMP), Colorimetric.	and are easy to fabricate,		
Nanomaterial based	Surface-plasmon resonance Labelled with gold nanoparticles, Single-walled carbon nanotubes (SWCNTs), and electrochemical.	easy to miniaturise, high electrical conductivity, magnetic properties, unique physiochemical properties, multiple uses, low detection limit, and integration in the smartphone.	Small size and large surface area can lead to particle aggregation, it still needs a skilled technician to understand the results.	[38-43]
Other biosensors	Electrochemical graphene, Magnetic resonance relaxometry, Optical spectroscopy	can offer an improvement to the quality of life in patients with chronic disease, detection of antibodies, monitoring blood-alcohol content levels, hemoglobin density measurements	Invasive (when monitoring blood samples), sensitive to the refractive index neighbouring the interface,	[38-44]

I.6- Thesis' Objective

The General Purpose of our research is:

- Make use of the powerful analytical capacities of the Point-of-Care Spectral Technology for blood clinical analysis parameter quantifications and disease classification.

The specific goals are:

- ✓ Make a comparison between the technology used by MINDRAY BC-5000 vet and the PoC photonic technology;
- ✓ Perform and observe the blood spectral fingerprint against the extreme values of the hemoglobin (HGB) and RBC to diagnose anemia;
- ✓ Perform HTC (dehydration control);
- ✓ Perform WBC (infection control);
- ✓ Perform spectrum processing to quantify parameters and classify hematological illness;
- ✓ Blind validation of results.

II. MATERIALS AND METHODS

Herein, I will present the main experimental methods, materials, spectroscopy technology, signal processing, and artificial intelligence methodologies for implementing the PoC hemogram analysis technology.

II.1- Samples´ Picking and Storage

The blood sample was picked by venepuncture in EDTA tubes [2] [36] from 155 healthy and ill dogs and 63 healthy and sick cats. To ensure the precision of hematologic interpretation and minimise the artifacts as much as possible, the blood samples were collected from the jugular vein by qualified personnel at the Centro Hospitalar Veterinário do Porto (CHVP), from March 2022 to June 2022. The samples´ quantities were analysed within 2 and 4 hours after collection. These samples were remainder used by Mindray BC-5000 vet hematology analyser [36].

II.2- PoC

PoC is one of the diagnostic systems, it is a technique that can rapidly test results allowing assisted patients to receive immediate treatment and this kind of system can be performed by non-expert persons, with few user actions.

The range spectrum is contained in Ultraviolet-Visible Near Infrared (UV-Vis-NIR) wavelengths, between 200-2500 nm wavelengths, specifically in 200-1200 nm wavelengths [29].

The technology used by PoC is reagent-less [2], and we do not have any preoccupation with calibration, because we need to use only a smartphone connected by the network of the PoC device. This kind of technology is highly sensitive. Therefore, the coefficient of variations in different yardsticks is practically null, warranting accuracy in results and avoiding false diagnostics.

In my thorough investigation, it is useful to highlight modularity, which is extremely useful for measurement between different patients or even if one patient needs several different body fluids to be analysed [29], in this case, the Mindray BC-5000 vet is not a better device to use.

One of the main focuses of this research is to improve the PoC system so that, may improve the state-of-the-art limitations. It can effectively provide real-time, non-invasive, non-destructive monitoring of a wide spectrum of relevant chemical parameters for clinical findings of diagnostic relevance and monitoring based on spectral fingerprinting at the PoC [29].

The possibility to get the spectral erroneous information taken by the PoC device is practically non-existent.

II.3- Spectrum Acquisition

To acquire the spectrum, I was following the simple steps to be possible: the first was to homogenise the sample with a slow movement in the EDTA tube where the sample was with a graduated pipette, measuring ~5 µl of the sample volume that I was putting in a capsule built with opposing mirrors [2], and slightly closed and docked in PoC device, wherein, the chamber that receives a sample of body fluid is characterised by an optical system comprehending by one source for emitting light in the sample; and a spectrometer for recording the spectrum of light from the sample, said light from the sample being of transmittance, reflectance, or Raman scattering of the emitted light by a said sample [29]. To optimise the spectrum acquisition parameters, the temperature of the LED was automatically controlled, the intensity of the LED was chosen to the highest level (1023 dB) and the integration time was regulated depending on the level of absorption that the said sample presented, therefore, this time was altered allowing to obtain the best spectrum and the information is stored in PoC device. The last step is to take out the capsule and sterilised it with deionised water, with the help of the piece of paper I dried up the capsule, and it was again prompt to take the spectrum of the other sample.

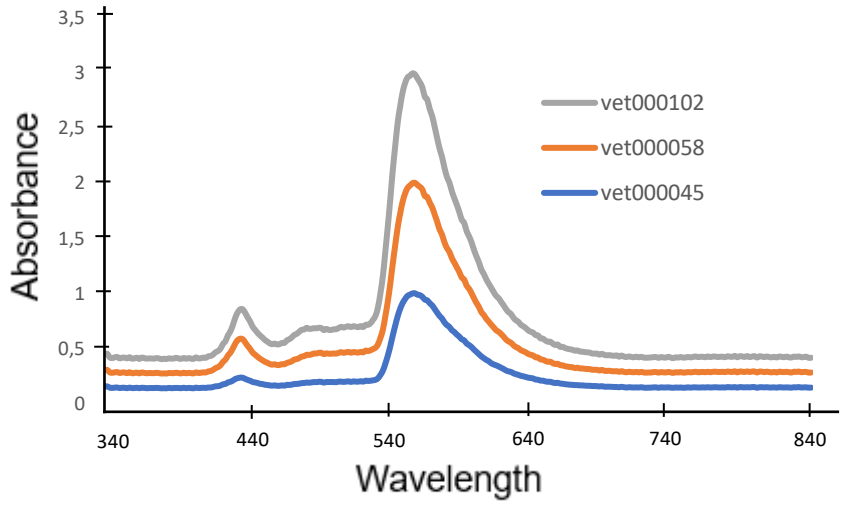
The figure below represents the moment that I was taking the spectrum of one sample.

Figure 2. PoC device: Spectrometer system controlled by IoT software in the mobile phone.

II.4- Data Organisation

The data was organised into 1. Hemogram data; and 2. Spectroscopy data.

Both were related to a key sample anonymised to keep the data confidentiality of the patient. In the dataset of hemogram, there is quantitative information about the following parameters: counting of Erythrocytes (RBC), quantification of the Hemoglobin (HGB), Hematocrits (HTC), the estimative of the mean corpuscular volume (MCV), and the mean corpuscular hemoglobin concentration (MCHC), counting of white blood cell (WBC), total platelets (PLT), as well as a percentage of populational qualitative of the WBC in terms of the Lymphocytes (Lymph) and monocytes (Mono). The spectral record of the intensity of every wavelength was organised in the table. Graph 1 represents the visual way to identify (by the human eye) the state of the health of some animals; on the graphic, we can see if the pet is normal, anemic, or polycythemic. It is possible to observe that dogs' blood in these cases exhibits higher absorbance in the hemoglobin bands region between 340 to 540 nm and lower in 540 to 840 nm.



Graphic 1. Dogs' spectrum shows three different levels of HGB. Low HGB (anemic), Normal HGB and High HGB (polycythemia).

II.5- Spectral Analysis Methods

II.5.1- Chemometric Methods

The following chemometric methods were tested and benchmarked for hemogram parameters quantifications using the PoC data:

- Linear models: i. multivariate linear model using both ferric and oxidised bands 541, 560, 576, and 628 nm; and ii. univariate linear model at the topmost peak of spectra (628 nm) [45, 46]. The linear regression was performed with the help of least-squares [47];
- Similarity: used the spectral and compositional similarity given by the Euclidean distance to several neighbouring samples in the principal components scores space to evaluate the composition, the number of neighbours optimised by cross-validation;
- Partial least squares (PLS): maximise the covariance between the spectra matrix X and blood composition matrix Y, for determining the eigenvectors of X^tY [48, 49]. The method forced the latent structures of spectra and composition (t and u) to be equal (NIPALS algorithm) [49], by the determination of this correspondent basis P^t and Q^t [48], where $X = TP^t$ and $X = UQ^t$. It outcome in deflation and ongoing orthogonal eigenvectors of the remaining information in X^tY [49, 50]. To optimise the number of latent variables (LV) I was using the cross-validation minimal predicted sum of squares. PLS were used at an oblique projection to determine the b_{pls} coefficients in the $Y = Xb_{pls}$ [49, 50].
- Local PLS (LocPLS) used sub-sampling of a principal component analysis (PCA) or PLS scores space to set up an ensemble of local PLS models [51]. The PCA scores were used to cluster the samples for each local PLS model. The number of PCA and PLS LV were optimised by cross-validation.

The PCA of hemogram composition and spectral information was performed to understand the information structure of the variance of these datasets and determine existing similar groups of samples with common disease classifications.

II.5.2- **MACHINE LEARNING METHODS**

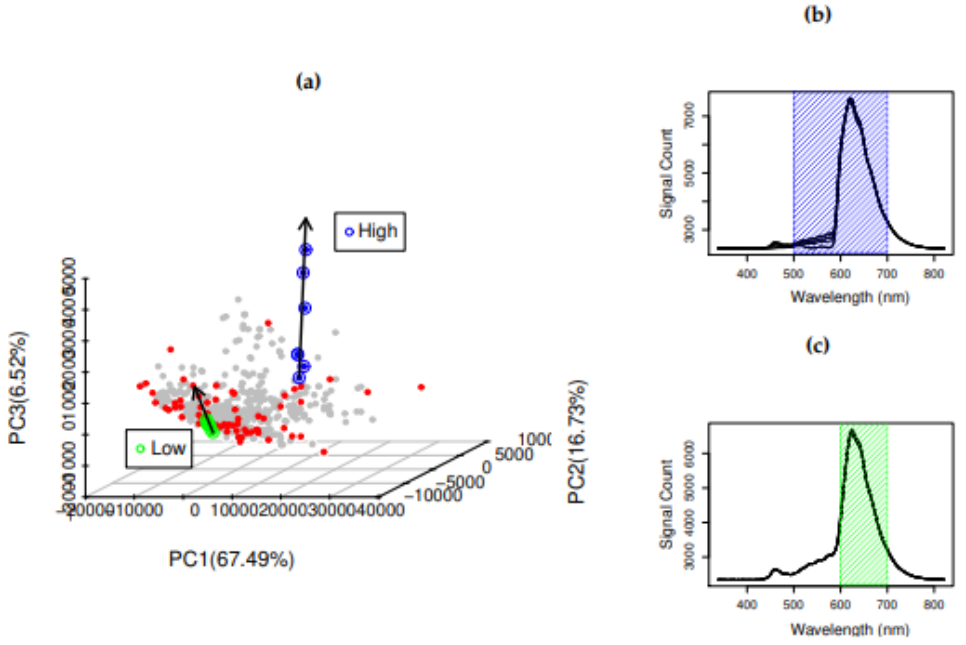
❖ Artificial neural networks (ANN): were introduced in spectroscopy as an approximate to deal with the non-linearity of spectral information (biological variability, multi-scale interference, and matrix effects). This method is a piecewise linear with activation functions at each neuron of the network, and its parameters are optimised by back-propagation. Most ANNs in spectroscopy use PCA or PLS punctuation as input,

being designated ANNPCA and ANNPLS [52, 53]. Tangent and identity functions were used as hidden and output layer activation; regressed by back-propagation using the Levenberg-Marquardt algorithm. The optimal number of PCA, PLS LV, and hidden layer architecture (number of layers and number of neurons per layer, e.g., genetic algorithm) was validated by cross-validation.

❖ Self-Learning Artificial Intelligence (SLAI): The basic principle of SLAI is the search for systematic and stable covariance between composition and spectral characteristics [54]. Stable covariance has a direct relationship to the BLL and SLAI, and I used this relationship to unscramble the complex multi-scale interference between blood constituents. Such is performed in two different steps:

1. To optimise the characteristic of the space: information about a constituent is present in the spectra in different scales and wavelengths. Selecting the correct feature and transforms (e.g., singular value decomposition, Fourier or Wavelets transforms) is useful to extract the information into a characteristic space that holds proportionality to the concentration of the constituents; and

2. Covariance mode (CovM) search: searching a group of samples within the feature space that belong to the same interference pattern, that is, a gradient mixture of interferents, where information is proportional to the constituent concentration. Such means that spectral features X hold the same information as composition Y, with a stable covariance X^tY.



Graphic 2. SLAI CovM demonstration: (a) high and low WBC CovMs in PCA scores space - ● mixture samples, ● real samples; ● CovM samples with low WBC; ● CovM samples with high WBC, and → the CovM vector; (b) High WBC CovM spectra and wavelength variance correlated to WBC (blue rectangle); (b) Low WBC CovM spectra and wavelength variance correlated to WBC (green rectangle).

The graphic 2a presents the score plot of the three first PC of the PCA of dog and cat spectra. PCA increases spectral variance by orthogonal single valuer decomposition of X. The scores space is spawned by three LVs, PC1 (67,49%), PC2 (16.73%), and PC3 (6,52%). These describe the principal sources of spectral variance, which in the case of blood, is the corresponding variance in RBC, Hgb, HTC, WBC, and interferences (e.g., Bilirubin). Dog and cat do not form independent groups, but cat samples are mostly positioned at higher values of PC1 than dog samples. This is because PC1 mostly represents the variance in RBC, Hgb, HTC, and WBC; PC2 and PC3 are interference information. Directions and distances in the scores space are expected to be proportional to changes in these yardsticks. Distance metrics (e.g. Euclidean or Mahalanobis) are commonly used as a measure of compositional similarity in LocPLS and Resemble methods [55, 56].

The CovM of each group has only one eigenvector, where the latent structure of spectral features and Hgb concentration are equivalent. It is further non-obvious to the human eye that the dog blood sample group (●) belongs to the same CovM. Despite the higher intensity of the spectra as the group (●), it belongs to the same CovM as the group (●) because sample projection is in the same eigenvector, being predictable by the CovM information as presented in the graphic. 2c. In this research, CovM search is performed in the PCA scores feature space by the local direction search and optimisation algorithm [54]: for a said region of the space characteristics, samples are clustered in different directions, by a pre-determined number of search directions in the spatial characteristics. From this first interaction, directions with high correlation and low LVs are stored. The search is further improved in these groups by maximising the covariance of sub-groups. This is performed by evolutionary methods (e.g. genetic algorithms), by adding and removing samples until a stable covariance group is found. The CovM is regularly established when one LV is sufficient for providing a small standard error, which is got by cross-validation. Once a small number of samples are in the CovM group, the leave-one-out cross-validation is used. This procedure is repeated for all remaining samples in the feature space until all CovMs are found. For samples that do not belong to a particular CovM, a non-good prediction is performed by searching for the best projection into the space of each CovM score. The prediction of a blind validation is performed by searching in which CovM the spectra projection is within the interpolation of the covariance eigenvector.

II.5.3- **Specific Methods for Spectroscopy**

The Vis-NIR spectra were pre-processed before SLAI, PLS, and LocPLS, to remove Vis-NIR artifacts effects of baseline shifts, Mie and Rayleigh scattering, and stray-light [57, 58]. The scattering coefficients were determined by regression against the median spectra of the dataset. Each corrected spectrum was obtained by applying the estimated coefficients to the extended inverse scatter correction (EISC) formula holding the spectra to be corrected [57].

II.6- Statistical Validation

The main goal of data-driven model optimisation is to capture systematic information, allowing to ensure that the model description of data is optimal, without under or overfitting. Cross-validation and hold-out samples guarantee a numerical equivalence to the null hypothesis test. The systematic information extracted by the model during cross-validation on the training dataset must be the same as the one contained in the hold-out sample. The threshold systematic information captured by each model is defined when the prediction errors of cross-validation are statistically like the hold-out samples. All models were optimised using random sampling cross-validation (95%) and tested using hold-out samples (5%) dataset splitting, being the performance metrics computed with the hold-out samples [59]. Case of the LocPLS and SLAI, cross-validation was performed using a leave-one-out and 1 hold-out scheme due to the lower number of samples at each local dataset cluster.

All models were benchmarked using the Pearson correlation coefficient (R) and mean absolute standard error percentage (MASEP). These relative error metrics were used to benchmark literature data. All computations were performed using the R project as statistical computing software (PLSR and NEURALNET packages; and LocPLS and SLAI) [60, 61].

III. DISCUSSION AND OUTCOMES

III.1- Make a Comparison Between the Technology Used by Mindray BC-5000 Vet and the PoC Photonic Technology

One of the goals of research was to validate the data compared with the data of the CHVP, and they have used Mindray BC-5000 vet, which is configured with the latest technologies to solve vet clinic troubles. It is useful to say, that the Mindray present to us a full result of the CBC even that, sometimes is possible to get an inconclusive result, however, this device needs different technologies to be possible:

For WBC differential count, it has been used flow cytometry combined with tri-angle laser scatter and chemical dye;

The RBC and PLT counts, in Mindray BC-5000 vet have been used electrical impedance;

And for HGB determination has been used calorimetric method, which is cyanide-free [36].

We can find in this device 3 routine reagents cyanide-free to maintain better working.

The technical specifications are WBC, Neu%, Lymph%, Mon%, Eos%, Bas%, Neu#, Lymph#, Mono#, Eos#, Bas#, RBC, Hgb, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, PDW, PCT, 3 Scattergrams for DIFF and 3 Histograms for WBC, RBC, and PLT.

Table 3. present the data of vet000183 gotten from CHVP.

sample vet000183									
Specie	RBC	HGB	HTC	MCV	MCHC	WBC	PLT	Lymph	Mono
Dog	5,40E+12	139	0,371	68,6	376	7,10E+9	2,54E+9	9,50E+9	4,40E+8

I think that it is important to highlight that this benchmark is about one product and one prototype. But this note does not reduce the significance of this work. The PoC device presents us with a spectral technology that is capable to determine CBC without joining with the other technologies is mostly impossible to present inconclusive results, because of its higher sensibility and this technology takes the vantage to be reagent-less.

The PoC device presents all the technical specifications that we can find in Mindray, however, this device is not a finished product. We are in the continuous process to train the machine, and for this reason, we do not present here the complete technical specification, but all the outcomes have been encouraging.

Table 4- present the spectral data of vet000183 taken from the PoC device.

sample vet000183									
Specie	RBC	HGB	HTC	MCV	MCHC	WBC	PLT	Lymph	Mono
Dog	5,80E+12	140	0,471	68,3	377	7,20E+9	2,74E+9	9,30E+9	4,70E+8

III.1.1- Technologies Limitations

I am conscious that we do not have anything around the world that is perfect, our technology is one of these cases. Besides the limitations that were presented in table 1, I will show other functional limitations.

Some limitations are independent of the technologies, because they depend on associated artifacts like: a poor collection of the sample (using incorrect methods and few or high samples quantities compared with Anticoagulants used in hematology), the way to carryover the sample, how to homogenise the sample, and how the sample is stored (frozen or heated) [62-64].

The embarrassment of these technologies is in fact that it was built for human samples and when these are generalised, in other species it is obvious to meet different characteristics and make it difficult to get a better result [63].

The inability to provide valuable reticulocyte or other precursor information, regularly correspond to an increase of erythrocytes or the type of hematologic population wherein this precursor belongs [63, 64].

Platelet clumping frequently results in an artifact (with a great incidence in cats) of increased total WBC or RBC count and decreased platelet counts because impedance counters often register clumped platelets as leukocytes or even erythrocytes [64]. Blood specimens from cats are known to clot quickly, and their platelets are frequently aggregated, so, when we get low platelets numbers good hematologic practices imply necessarily evaluating the blood smear [63, 64].

We find high imprecision when evaluating the WBC differential due to morphologic alterations and in this case, the manual count should be reported [65]. Is useful to highlight that, in this research, I have presented only the lymphocytes and monocytes.

What allowed me to evaluate the performance of the hematologic devices that use different technologies was the concepts presented by ASVCP, about the allowable total error (TEa) and observable total error (TEobs).

The reference laboratory requires that the TEobs must be lower than the TEa, its ideal condition to assure the reliability of results of hematologic parameters [63, 65-67].

Table 5 shows the difference between TEa presented by standard laboratory and TEobs that we get from our technology.

Table 5. Values that evaluate the performance level of the technology

	Standard Laboratory	Spectroscopy PoC
Measured	TEa	TEobs
RBC	10%	17%
Hgb	10%	17%
HCT	10%	17%
MCV	7%	13%
MCHC	10%	17%
PLT	20% à 25%	37%
WBC	15% à 20%	21%, 40%
Lymph	15%	25%
Mono	60% (within RI) and 50% (above RI)	60%

It is also useful to highlight that, the optimal standard value, at least until this moment we do not have any technique capable to achieve these results, therefore, we assume that this analysis must be followed by additional tests that may be required to take a better conclusion (smear blood) [63, 65, 66].

III.1.2- Using Spectroscopy PoC Photonic Technology in **Low-Income countries** (case of Angola)

The feasibility of medical exams for the population as a whole is particularly important in developing countries. They have many difficulties to make functional the current western systems. Because these countries have been living with many troubles, such as the lack of electric current in many parts, the low number of specialised professionals, and the lack of structure to put these instruments working rather a decentralised health system such as PoC offers to allow us to have the most effective

means. Therefore, a decentralised diagnostic process with PoC systems can change the living reality in Angola. There have been a few successful movements in this direction [68]. Furthermore, widespread use of the currently available systems is precluded by costs, complexity, and unreliability under extreme conditions of heat or humidity. The yardsticks offered often do not meet those required in these situations. The American National Institute of Biomedical Imaging and Bioengineering (NIBIB) has therefore begun cooperation with India, the aim of which is to develop devices, which are suitable for use at the point of need [69]. The International Council for Standardization in Hematology (ICSH) is working on a set of guidelines, which will be applicable worldwide for PoC standardization in hematology and can also be used in low-income countries [70].

Early diagnosis is essential to reduce mortality and interrupt transmission, but the insufficient healthcare infrastructure in developing countries limits their accessibility and effect. The device is applicable in rural areas without being compromised by a lack of analytical quality [71].

The ASVCP assumes that good hematologic practices should be followed by quality control, so, I have the notion that in the case of Angola we do not have the possibilities to do it. It means that between doing it and avoiding it, I prefer trusting in the results of our technology.

III.2- Perform and Observe the Blood Spectral Fingerprint Against the Extreme Values of the Hemoglobin (Hgb) and RBC to Diagnose Anemia

Evaluating the RBCs (erythrogram) RBC data include the PCV, RBC count, hemoglobin concentration, mean cell volume, and mean cell hemoglobin concentration. we include total protein concentration as part of the RBC evaluation [64]. The RBC data are used to determine whether the RBC mass is normal, reduced, or increased as we can see in graphic 1. RBC mass is evaluated by assure the PCV, hemoglobin concentration, and RBC count. PoC test, one reason for determining all three is to achieve some measure of internal quality control. Any deviation from the reference range should occur in all three tests to the same degree. If the results of the three tests do not correlate, consider the possibility of laboratory error.

Multivariate linear models using hemoglobin band models (Hgb bands) provide a moderate relationship between transmittance and RBC, Hgb and HTC (R ~ 0.57–0.61), and a strong correlation in cats (R ~ 0.91–0.94) (p-value < 10^{-3}). Dog blood optical properties are strongly reduced in the hemoglobin bands' linearity with RBC, Hgb, and

HTC quantifications. This is because of the significantly lower signal variance in the Hgb bands. For this reason, a simpler univariate linear model of transmittance at 628 nm is unable to provide a valid correlation in dogs (R ~ 0.29–0.32) and only provides a moderate correlation in cats (R ~ 0.61–0.63). Quantification using the similarity method was optimised to 4 neighbours in the PCA scores space (graphic. 2), giving moderate correlations for RBC, Hgb, and HTC for dog hemograms (R ~ 0.64), and significant correlations for cat (R ~ 0.87–0.89). This inconsistency is because Euclidean distances in the PCA space do not directly correspond to solely RBC, Hgb, or HTC, as punctuation corresponds to spectral variance with more contribution in the region of 600 to 750 nm. Spectral variance (X^tY) is not directly related to hemogram covariance (X^tY) due to multi-scale interferences. PLS quantification has significant correlations (p-value < 10^{-3}). for RBC, Hgb and HTC for the dog (R ~ 0.86–0.87) and cat (R ~ 0.92–0.94), using 5 and 3 LV. The number of LV has major implications in the SLAI method and has very significant correlations for the dog (R ~ 0.93–0.95) and cat (R ~ 0.98–0.99, p-value < 10^{-3}) hemograms. SLAI can reduce the dimensionality to 1 LV in most of the dog and cat datasets for quantifying RBC, Hgb, and HTC. In the case of samples that do not belong to a particular CovM, it uses 2 LV.

SLAI uses 1LV in optimal CovM, and more LV to compute the predictions for incomplete CovM samples, allowing acceptable performance even in incomplete datasets. The CovM holds the BLL relationship for a group of samples, showing the same type of interference. Supplementary material presents the relationship between the BLL and CovM from SLAI, providing reasoning on how to unscramble complex multi-scaled information present in spectral data. The CovM can be regarded as the interference fingerprint of a particular set of constituents in blood spectra, where t $\simeq$ u. This observation is corroborated by the high statistical stability of the CovM eigenvector using cross-validation, which means that each of the samples can always be predicted by the systematic information of the mode, and the first eigenvector must hold the property: $X^tY_{n-k} \simeq X^tY_n$ [54], where n is the total number of samples of a particular CovM, and k is the number of samples from the CovM left out for validation. This property allows for determining if a particular hold-out sample belongs to a CovM and can be predicted [54].

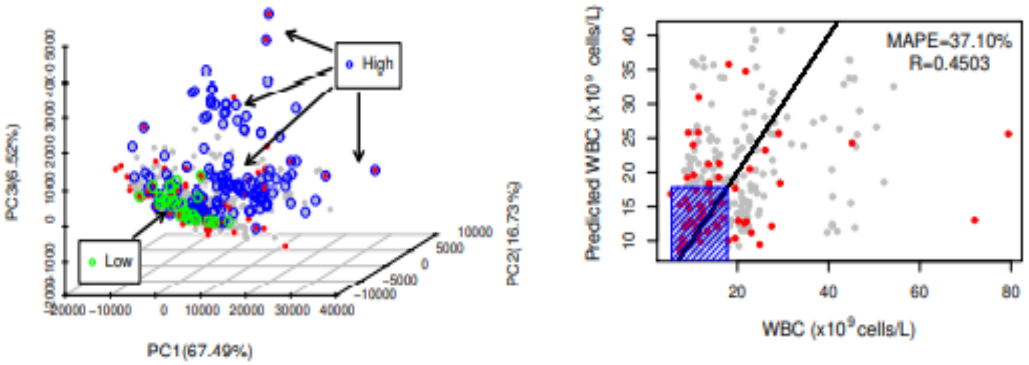
III.3- Perform HTC (dehydration control)

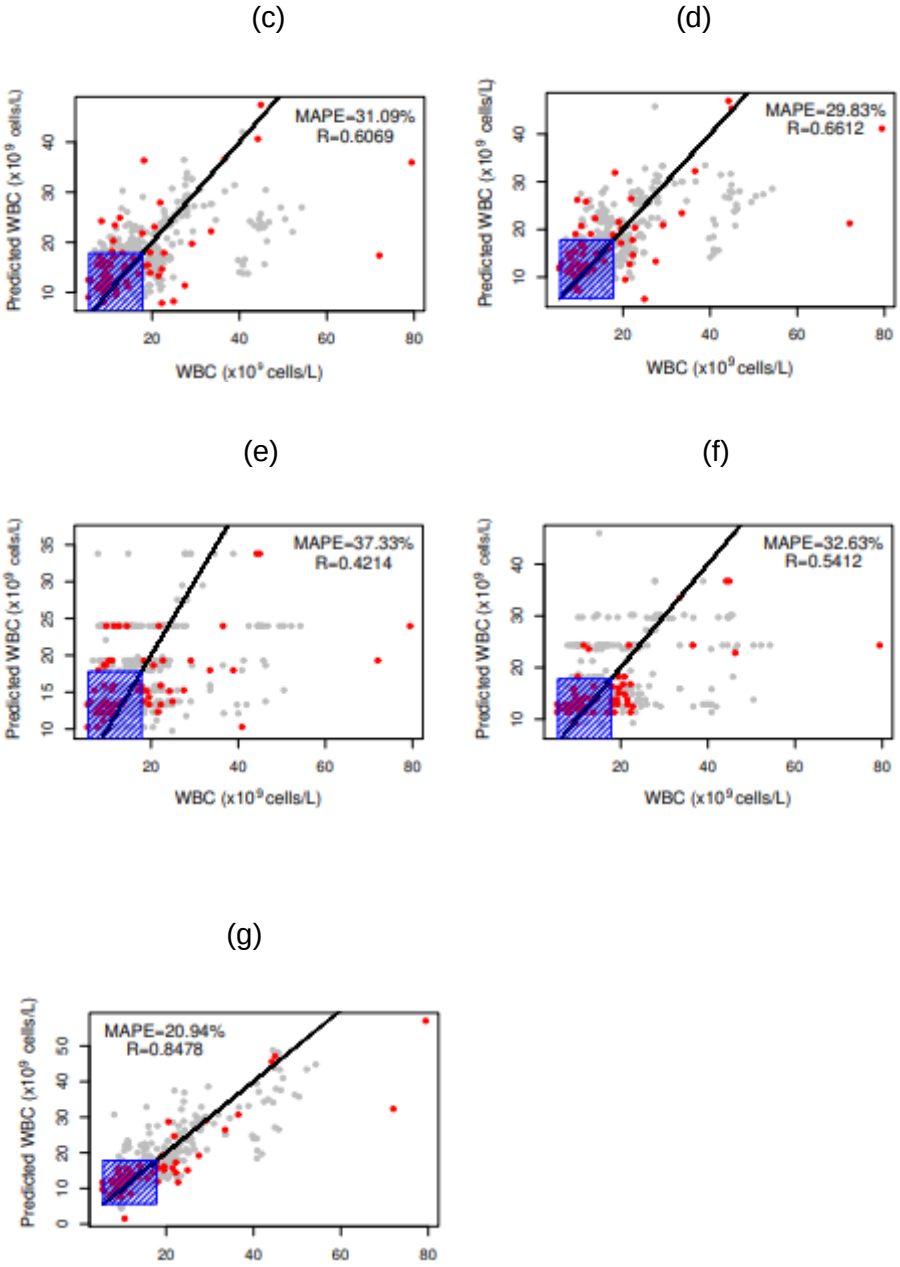
If erythrocyte mass is increased, then the animal is polycythemic as we can see in the low line of the graphic 1. Is the polycythemia relative or absolute? Relative polycythemia, the most common polycythemia recognised in veterinary medicine, is the result of hemoconcentration [64]. Relative polycythemia is generally established based on the clinical history and signs consistent with dehydration, as well as an elevated total protein concentration. Polycythemia in the absence of these findings is absolute. Absolute polycythemia can be either primary or secondary. Primary absolute polycythemia, also called polycythemia vera, is a myeloproliferative disease. It is a multipotential stem cell defect characterised by the overproduction of all cell lines. The clinical signs—including lethargy, hyperemia, dyspnea, cardiac murmur, and splenomegaly—are attributed to hyperviscosity syndrome related primarily to increased RBC mass. A markedly increased RBC mass (e.g. PCV = 75%) in the presence of normal arterial oxygen concentrations and normal or low serum erythropoietin concentrations generally confirms the diagnosis. Secondary absolute polycythemia is both appropriate and compensatory for hypoxemia or inappropriate and caused by increased concentrations of circulating erythropoietin. Compensatory polycythemia is present in animals recently moved to high altitudes or that suffer from pulmonary or cardiovascular disease. Inappropriate secondary polycythemia, characterised by excessive erythropoietin production, often accompanies renal carcinoma, renal cysts, hydronephrosis, and, occasionally, nonrenal tumours [64].

III.4- Perform WBC (infection control)

SIM was optimised using 3 neighbouring samples, taking the Euclidean distance in the 3 PC scores space, totalising 90.74% of the spectral variance [graphic 3 (a)].

(a) (b)





Graphic 3. (a) WBC spectral information of the PCA scores of blood spectra, WBC prediction for: (b) SIM; (c) PLS; (d) LocPLS; (e) PCA-ANN; (f) PLS-ANN and (g) SLAI. where: ◯ mixture of hemogram/spectra samples, ● blood samples, ● low WBC, and ● high WBC; → hemogram PCA loading.

SIM has a low correlation and high error values (R=0.4503, MAPE=37.10%) (Table 6, graphic. b). There is a high discrepancy between real and mixture datasets in terms of Pearson correlation coefficient (R). This low performance is because the Euclidean distance in the T space does not directly correspond solely to WBC information and spectral variance.

Table 6. WBC quantification metrics using Mixture and Real datasets.

Methods	Parameters	Dataset	R	MAPE (%)
SIM	nPC=3	Mixture	0,5005	35,66
	n=3	Real	0,4503	37,10
PLS	LV=6	Mixture	0,6069	27,66
		Real	0,6038	31,09
LocPLS	LV=5	Mixture	0,6810	26,51
		Real	0,6612	29,83
PCA-ANN	LV=3	Mixture	0,4797	33,85
		Real	0,4214	37,33
PLS-ANN	LV=3	Mixture	0,5910	28,79
		Real	0,5412	32,63
SLAI	LV=1	Mixture	0,9032	15,57
	nCovM=100	Real	0,8478	20,94

n - number of neighbours; nCovM - number of CovMs.

(X^tX) is not directly related to covariance (X^tY). Results for spectral PoC hemogram of RBC, Hgb, and HTC [56], also demonstrated that spectral similarity cannot represent the first principles of the BLL. PLS has a significant correlation (R=0.6038), but high prediction errors (MAPE=31.09%) (Table 6, graphic 3. c). The Pearson correlation for real (R=0.6038) and mixture (R=0.6069) datasets is similar, but PLS has very different error performances between the two datasets (MAPE of 31.09% and 27.66%, respectively) (Table 6). PLS model was obtained using 6 LVs. The high number of LVs has implications in the interpretation of the PLS coefficients, By adding new dimensions, more interferences are accounted for WBC quantification, resulting in a weighted oblique projection of all existing covariance modes [56, 72].

As the eigenstructure of X is similar to Y, the PLS algorithm is capable to converge into an acceptable correlation value. As there are many types of spectral gradients due to interference, PLS is not capable to take into account the details of each CovM. PLS is extremely effective when the global covariance (X^tY) is stable, that is when interference is restricted to a small number of CovMs, where the variance of samples is not complex (e.g. high purity chemical product), which is not the case of blood samples. PLS shows that there is a global correlation between spectral information and WBC. The smaller scale of variance in spectroscopy signals due to WBC concerning RBC and Hgb implies that the PLS model needs high dimensionality (6 LVs) to best represent the information. PLS is unable to further increase the dimensionality without overfitting, because many CoMs do not share the same RoIs used to quantify WBC.

LocPLS has better performance than PLS, with an R=0.6612 and MAPE=29.83% (Table 6, graphic 3. d). It also has a good correlation agreement between real (R=0.6612) and mixture datasets (R=0.6810), but significant differences in terms of MAPE values (29,83% and 26.51%) (Table 6). LocPLS breaks down the complexity of the global covariance (X^tY) into an ensemble of PLS models along the spectral variance space T, considering that a subset of similar samples may hold stable covariance. It was also expected a significant reduction in the dimensionality of the PLS models resulted in a negligible decrease to 5 LVs (see Table 6), and no significant gains in correlation (R) or prediction errors (MAPE) compared to PLS. LocPLS does not perform a systematic search for stable covariance but it uses similarity metrics (Euclidean distance) to group samples. These may or may not belong to the same CovM, resulting in a non-systematic dimension reduction and model performance. LocPLS efficiency is higher in blood constituents that have dominant information in the spectra (e.g. RBC, Hgb, and HTC) [56], not being effective with non-dominant constituents, such as WBC.

SIM, PLS, and LocPLS cannot model extremely high values of WBC, which have outlier characteristics to the rest of the datasets. Two extreme groups with WBC in the range of 40 to 70 × 10^9 cells/L are outliers to the main model (graphic 3. b to d). The high dimensionality of PLS and LocPLS (6 and 5 LVs, respectively) does not capture the CovM to which these samples should be associated to predict WBC accurately. ANN (PCA-ANN and PLS-ANN) exhibits low performance when modeling WBC spectral information, compared to SIM, PLS, and LocPLS. The Pearson correlation (R) is 0.4214 and 0.5412 for PCA-ANN and PLS-ANN. Prediction errors are high, with a MAPE of 37.33% and 32.63% for PCA-ANN and PLS-ANN (Table 6, graphic 3. e). Both ANN models were optimised with 3 LVs and architecture of 3 hidden layers (Table 6). The performance of ANN models is consistent between the real and mixture datasets, showing the information is similar between datasets, obtaining the same level of performance. PLS-ANN has a better performance than PCA-ANN because PLS scores are obtained by maximising the covariance, whereas PCA maximises the variance of the spectral datasets. ANN has high difficulty in finding consistent covariance, especially with low levels of spectral variance of WBC. As ANN is designed using piecewise linear and activation functions, they have better performance mapping non-linear phenomena to which there are clear decision boundaries between classes.

PCA-ANN and PLS-ANN showed satisfactory performances only when modeling dominant spectral information, such as RBC, Hgb, and HTC [56], ANN approaches struggle to cope with multi-scale interference of non-dominant blood constituents, such as WBC. SLAI presents significant correlations (R=0.8478) and low prediction errors (MAPE=20.94) (Table 6, graphic 3. g). Furthermore, it also has results between real and

mixture datasets: i. R: 0.8478 and 0.9032; and ii. MAPE: 20.94% and 15,57% respectively. SLAI reduces the dimensionality to 1 LV, being able to determine 100 CovMs among the two datasets. The capacity to model extreme values of WBC is significantly improved, where WBC levels between 40 and 70 ×10E9 cells/L are predicted with significantly less error, allowing to correctly diagnose of high levels of WBC.

III.5- Perform Spectrum Processing to Quantify Parameters and classify Hematological illness

The principal goal of the PoC is to analyse the Hematologic parameters for solving problems about blood disease. Despite the several approaches to minimise interference systematic effects persists in optical biosensors. Our application of signal processing, chemometrics, and artificial intelligence in biosensors has greatly improved the accuracy of the existing technology, by performing signal corrections and pattern recognition that hold quantitative information that we can find in blood [73, 74]. The capacity to unscramble both matrix effects and multi-scale interference in spectroscopy is a powerful strategy for optical biosensing, moving towards reagent-less point-of-care (PoC) systems [29, 54]. And our appliance was so effective because we understood that we were searching with relative facility.

Several technologies have been applied to determine erythrocyte, leukocyte, and thrombocyte counts in veterinary medicine the spectroscopy PoC technology is one of them.

CBC provides an overview of a patient's general health. For this reason, hematology is a critical component of the laboratory evaluation of patients. And our mission in this work is to improve and give better tools to facilitate the work of veterinarian Doctors.

The peripheral blood serves as the carryover medium between the bone marrow and the tissues; consequently, CBC provides immediate to the hematopoietic system at a specific point in time [64].

With spectrum processing get by PoC device we were capable to identify illnesses like:

evidence of inflammation when the WBC is increased, decreased Lymphocytes, and variable Mono;

evidence of a glucocorticoid (stress) the WBC is increased, the Lymph is decreased, and Mono is increased or unchangeable;

epinephrine (excitement) response the WBC is increased, and the Lymph and Mono are unchangeable. But in cats we have particularity in Lymph because this condition happens increased too;

the existence of demand of phagocytosis or evidence of tissue necrosis Monocytosis indicates this existence.

III.7- Blind Validation of Results

Here I analysed the level of operationality of the PoC device. I used some unknown samples.

And in this test, a performance test of our equipment was successfully performed, because the result was not different compared, to the result that they got using the Mindray BC-5000 vet. As we can see in the tables below.

Table 7. presents data from an unknown sample taken from the Mindray ab-5000 vet.

Unknown sample									
Specie	RBC	Hgb	HTC	MCV	MCHC	WBC	PLT	Lymph	Mono
Dog	4,44E+12	122	0,321	72,3	380	1,11E+10	4,82E+11	3,00E+8	2,65E+9

The result is really obvious and does not leave any doubts about the assurance and efficiency of this new technology.

Table 8. presents the spectral data of an unknown sample taken from the PoC device.

Unknown sample									
Specie	RBC	HGB	HTC	MCV	MCHC	WBC	PLT	Lymph	Mono
Dog	4,67E+12	126	0,331	71,9	378	1,21E+10	4,90E+11	2,99E+8	2,61E+9

IV. CONCLUSION

The work presented here appears to join in the effort to give more contributions to veterinarian medicine, and I am sure that in a short period of time this technology will be spread along the world in the area of medicine (vet and human). The objective of this research was to Make use of the powerful analytical capacities of the Point-of-Care Spectral Technology for blood clinical analysis parameter quantifications and disease classification.

The incapacity of the current chemometric methods to deal with the complex interference in blood samples, the inconsistency to get a better result, as well as to attain the error standards presented by ASVCP the use of many kinds of reagents, and the quantity used for the blood sample, make the PoC device a good alternative to improve the way of the lifestyle of our patients.

This work presents the veterinary community with a variety of news and most importantly the capacity of explaining the interference in the interpretation of the covariance modes coefficients, the capacity to determine with blind validation, and the capacity to provide results in real-time with a single drop of blood (~5µl) and the reagent less, makes the technology presented here unique in the field do vet.

The SLAI and CovM search algorithms showed themselves to be enabling technology to improve the current state of spectroscopy technology in the chemical sensing of a complex biological sample.

The result that has been gotten here improved the results of the state-of-art. But even so, it stays far away from the result presented by ASVCP: RBC (10%) 17%, Hgb (10%) 17%, HCT (10%) 17%, MCV (7%) 13%, MCHC (10%) 17%, PLT (20-25%) 37%, WBC (15-20%) 21%-40%, Lymph (15%) 25%, Mono (50%-60%) 60%. The result presented by ASVCP is an ideal result, but so far, we still do not have any technique capable of achieving this result. And the technology presented here is one that is closer to this reality. But this work is open for further investigation.

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