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Visible–Near-Infrared Platelets Count: Towards Thrombocytosis Point-of-Care Diagnosis †

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Abstract: Thrombocytosis is a disorder with an excessive number of platelets in the blood, where total platelet counts (TPC) are crucial for diagnosis. This condition predisposes to blood vessels clotting and diseases such as stroke or heart attack. TPC is generally performed at the laboratory by flow cytometry with laser scattering or impedance detection. Due to the limited capacity of automated hematology in performing TPC quantification, a manual microscopy count is a very common quality assurance measure undertaken by clinical pathologists. Monitoring coagulation risk is key in many health conditions, and point-of-care platforms would simplify this procedure by taking platelet counts to the bedside. Spectroscopy has high potential for reagent-less point-of-care miniaturized technologies. However, platelets are difficult to detect in blood by standard spectroscopy analysis, due to their small size, low number when compared to red blood cells, and low spectral contrast to hemoglobin. In this exploratory research, we show that it is possible to perform TPC by advanced spectroscopy analysis, using a new processing methodology based on self-learning artificial intelligence. The results show that TPC can be measured by visible–near-infrared spectroscopy above the standard error limit of 61.19 $\times$ 10^9 cells/L (R^2 = 0.7016), tested within the data range of 53 $\times$ 10^9 to 860 $\times$ 10^9 cells/L of dog blood. These results open the possibility for using spectroscopy as a diagnostic technology for the detection of high levels of platelets directly in whole blood, towards the rapid diagnosis of thrombocytosis and stroke prevention.

Keywords: point-of-care; spectroscopy; platelets; artificial intelligence

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1. Introduction

Platelets (PLT) are the smallest cells in the blood, being responsible for coagulation and blood vessel repair. The PLT counts reference interval in dogs is 300 to 500 $\times$ 10^9 cell/L. High PLT counts is a condition known as thrombocytosis, being attributed to abnormal bone marrow production or an ongoing condition such as anemia or inflammation [1]. Thrombocytosis can result in blood clots, leading to life-threatening or impairing conditions such as heart attack or stroke [2]. Automated PLT counts are mostly performed by flow cytometry, electric impedance (Coulter principle), or laser-scattering technologies [3]. However, these methods are prone to erroneous PLT counts, because of changes in cell size and morphology, due to blood clotting, activation, aggregation, or even post-sampling artifacts. This limits scattering angle and impedance detection, leading to misidentification

as larger cells, such as erythrocytes or leucocytes. Laser scattering is significantly more accurate than electric impedance, but the latter is cheaper and has a higher implementation in veterinary medicine. Veterinary doctors make use of blood-smear PLT manual counts for ensuring results quality in abnormal (low or high) values [4].

Visible-shortwave–near-infrared (Vis–NIR) spectroscopy has a high potential for the development of point-of-care (POC) without the need for reagents or complex sample preparation. The developed Vis–SWNIR POC system (Figure 1b) records the blood spectra of a single drop of blood (<10 µL) to provide a significant number of clinical analysis parameters with real-time results [5].

Visible-short-wave–near-infrared (Vis–SWNIR) spectroscopy is an information-rich technology that carries both physical and chemical information, where the information about blood cells and constituents is distributed across the different wavelengths. Dominant spectral information in blood comes from highly absorbent constituents in the Vis–SWNIR region, such as hemoglobin present in red blood cells (RBC) and bilirubin in blood serum.

Platelets are present in significantly lower values than red blood cells (RBC) (Figure 1a). The PLT reference interval in dogs is 300 to 500 $\times$ 10^9 cells/L and RBC is 5500 to 8500 $\times$ 10^9 cells/L, being at approximately 1:18 ratio to RBC, which makes the detection difficult:

i. Smaller size of PLT with the significantly lower area and volume for light absorbance, resulting in low sensitivity in the spectral signal;
ii. High interference between PLT and RBC and hemoglobin and bilirubin, which leads to the existence of significantly different characteristic interferences;
iii. High variance of PLT morphology—which can vary from small platelets to activated platelets with branches and clotted cells.

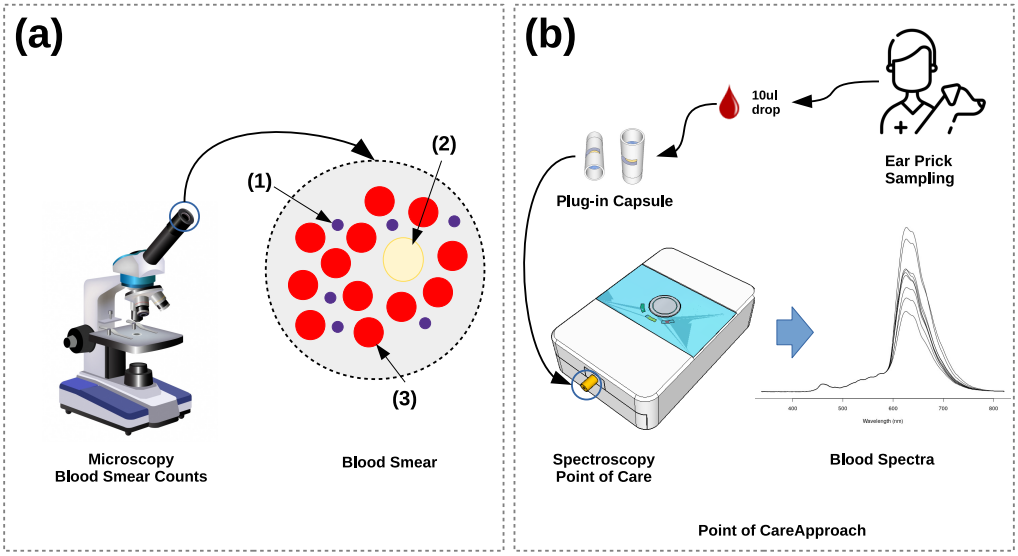

Figure 1. Platelets cell counts: (**a**) manual smear count at the microscope by trained hematologist demonstrating the proportionality between (1) platelets, (2) white blood cells, and (3) red blood cells and (**b**) point-of-care approach—single-blood-drop spectroscopy counts using artificial intelligence.

PLT counts are difficult to obtain, even by microscopy methods, exhibiting high variability. Herein, we explore the capacity of Vis–SWNIR and self-learning artificial intelligence (SL-AI) for PLT quantification [5]. This new approach isolates spectral interference by searching consistent covariance between PLT and spectral features, which belong to a covariance mode (CovM). CovM is a set of samples that can hold a direct relationship between spectral features and PLT counts, by sharing a common latent structure [5]. Ideally, PLT counts are related to spectral-interference features by a single latent variable (LV) or eigenvector. This allows unscrambling the interference of PLT concerning the other blood

constituents. This research provides a feasibility benchmark between the widely used chemometrics partial least squares (PLS) method and the SL-AI method.

2. Materials and Methods

2.1. Hemogram Analysis

Dog blood samples from routine clinical practice were collected by qualified personnel by standard venipuncture, at the Centro Hopitalar Veterinário do Porto. No animal experimentation was involved or any additional procedure. Samples used in this study are remnants from already necessary routine clinical analysis medical practice. Dataset is anonymized. PLT was determined by Beckman–Coulter capillary impedance using a Mindray B-2800 vet auto-hematology analyzer (Mindray, Shenzen, China).

2.2. Spectroscopy

Blood spectra were recorded using a POC prototype (INESC TEC, Porto, Portugal) using a 4500 K power LED as a light source and a USB-based miniaturized spectrometer (Ocean Insight STS-vis, Orlando, FL, USA), with an optical configuration and plug-in capsule system according to [6]. LED temperature and spectrometer integration times were automatically managed to maintain result consistency. Three replicate measurements were made for each blood sample.

2.3. Chemometrics

Spectral records were subjected to scattering correction (Mie and Rayleigh) before modeling. A feasibility benchmark was performed between PLS and SL-AI methods. PLS maximizes the global covariance between spectral features and PLT, by determining the orthogonal eigenvectors of the covariance matrix. The relationship between PLT and signal features is derived by the latent variables (LV), at each deflation. The number of LV is determined by cross-validation at the minimum value of the predicted residuals sum of squares (PRESS) [7].

SL-AI searches for stable covariance in spectral datasets, finding covariance modes (CovM). CovM is a group of samples that contains the same interference information characteristics, holding proportionality between PLT and spectral features. Ideally, the relationship between PLT and spectral features is given by a single eigenvector or latent variable (LV). The CovM is validated by leave-one-out cross-validation [5].

3. Results and Discussion

The PLS model attained a correlation of 0.2613 with a very poor R^2 (0.068) and a corresponding high SE of 175.99 $\times$ 10^9 cells/L. The PLS analysis shows that the correlation between spectral features and PLT counts is highly unstable and non-linear. Such is because PLT is present in much fewer quantities than other blood constituents (Figure 1), as well as, due to the small size and high interference with the other major blood constituents (e.g., RBC, hemoglobin, and bilirubin). Another indication of non-linearity is that the PLS algorithm attains the optimum prediction error with two LV, resulting in a non-significant model (Figure 2a). The PLS is unable to increase the number of LV because the information about PLT is scattered in significantly different interference modes that cannot be collapsed into a linear oblique projection model [5,7]. PLS cannot be used in a POC as it does not attain a MAPE similar to 25%—the total allowable error established by the American Society for Veterinary Clinical Pathology (ASVCP) for PLT counts [8].

SL-AI presented a significant correlation of 0.8376, an SE of 61.19 $\times$ 10^9 cell/L, and a MAPE of 24.67%, with R^2 of 0.7016 (Table 1). SL-AI covariance modes (CovM) were obtained with 1 to 3 LV. This means that, although statistically valid relationships were obtained for each CovM, some of these were integrating more than one type of interference. Under ideal conditions, all CovM should have only one LV, directly relating PLT counts and spectral interference.

Table 1. PLS and SL-AI benchmark results.

Method	SE	LV	R^2	MAPE (%)	$R_{Pearson}$
PLS	175.99	2	0.068	88.89	0.2613
SL-AI	61.19	1-3	0.7016	24.67	0.8376

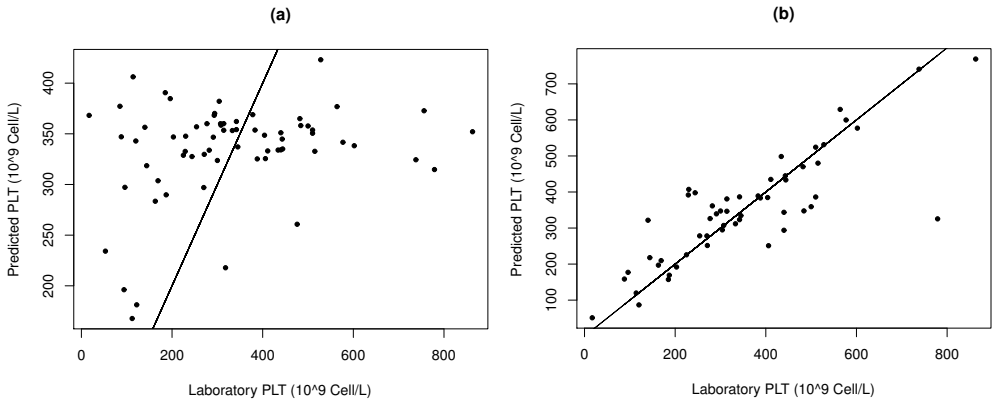

Figure 2. Total platelet counts spectral quantification: (**a**) PLS and (**b**) SL-AI.

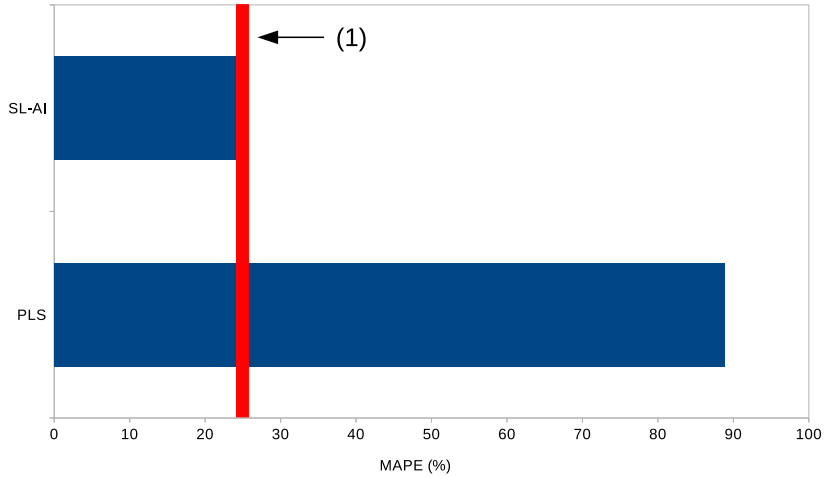

Figure 3. Percentage total error for PLS and SL-AI predictions: (1) ASVCP acceptable error limit (25%).

The results also show that non-dominant spectral information and low-scale spectral variation is unscrambled by the CovM principle. The number of LV can be attributed to the high diversity of PLT morphology present in dog blood (non-activated, activated, and clotted PLT) and the particular conditions of the tested blood, with correspondence in the major constituents.

Despite the limitations shown in this feasibility study, PLT quantification using Vis–SWNIR spectroscopy in conjunction with the new SL-AI algorithm can attain a total error estimate of 25%. Such a result is following the ASVCP total allowable error for PLT in dog blood [8] (Figure 3).

Vis–SWNIR POC technology based on SL-AI has shown high potential for PLT quantification and thrombocytosis diagnosis. The results presented for dog blood are within the acceptable error defined by the ASVCP of 25% [8]. The presented results also allow extending the potential application to both human and other animal species in further studies.

4. Conclusions

This feasibility study showed that low intensity, non-dominant, and multi-scale interferent spectral information is possible to be accessed, by unscrambling information with the CovM principle included in the SL-AI method. The small variations in the spectral signal

that contain information about PLT cannot be modeled by PLS. SL-AI can unscramble PLT interference information based on the CovM principle, allowing the quantification of PLT. Future studies, with more samples, may provide better insights on the full potential of the developed POC technology in both veterinary and human medicine.

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