

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

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**Abstract:** Thrombocytosis is a disorder with excessive number of platelets in the blood, being total platelet counts (TPC) 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 limited capacity of automated hematology in performing TPC quantification, 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. 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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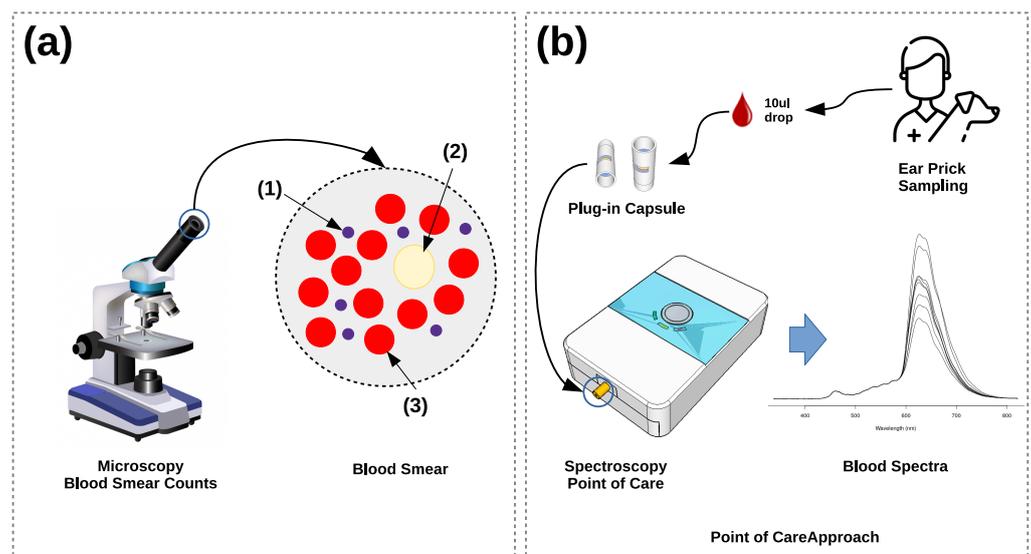
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## 1. Introduction

Platelets (PLT) are the smallest cells in the blood, being responsible for coagulation and blood vessel repair. 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



**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.

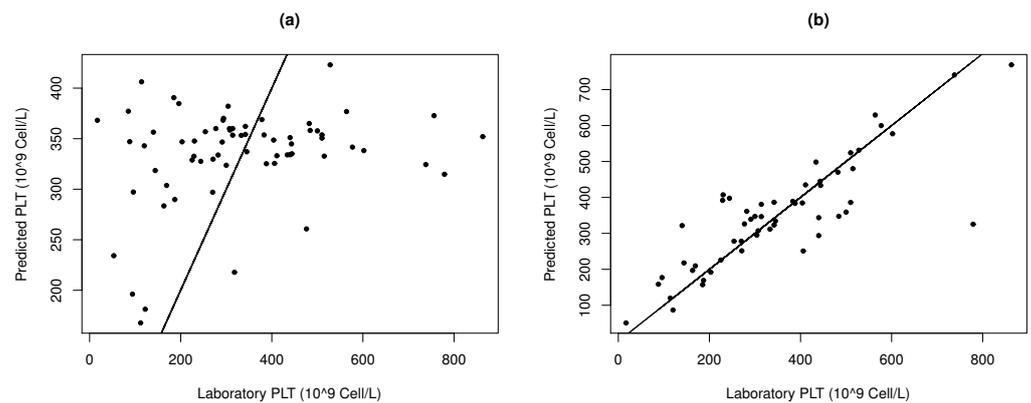
37 preparation. The developed Vis-SWNIR POC system (Figure 1b) records the blood  
 38 spectra of a single drop of blood ( $<10 \mu\text{L}$ ) to provide a significant number of clinical  
 39 analysis parameters with real-time results [5].

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

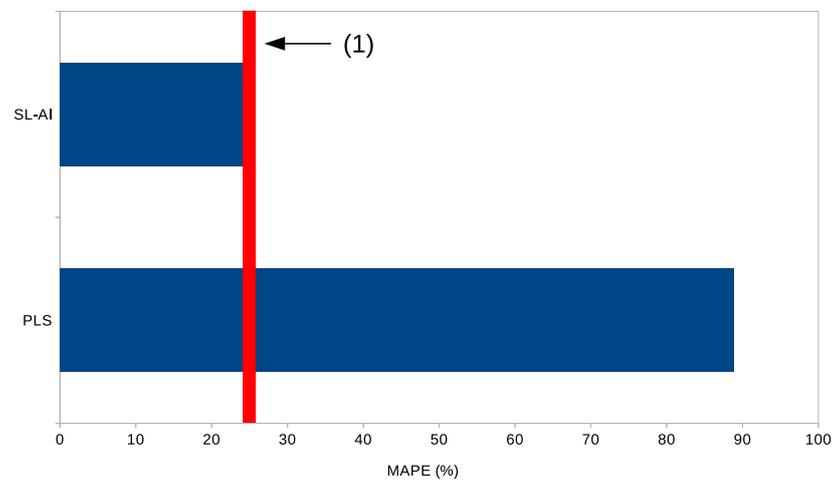
46 Platelets are present in significantly lower values than red blood cells (RBC) (Figure  
 47 1a). PLT reference interval in dogs is  $300$  to  $500 \times 10^9$  cells/L and RBC is  $5500$  to  $8500$   
 48  $\times 10^9$  cells/L, being at approximately 1:18 ratio to RBC, which difficults the detection:

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

55 PLT counts are difficult to obtain, even by microscopy methods, exhibiting high  
 56 variability. Herein, we explore the capacity of Vis-SWNIR and self-learning artificial  
 57 intelligence (SL-AI) for PLT quantification [5]. This new approach isolates spectral  
 58 interference by searching consistent covariance between PLT and spectral features,  
 59 which belong to a covariance mode (CovM). CovM is a set of samples that can hold  
 60 a direct relationship between spectral features and PLT counts, by sharing a common  
 61 latent structure [5]. Ideally, PLT counts are related to spectral interference features by  
 62 a single latent variable (LV) or eigenvector. Such allows unscrambling the interference  
 63 of PLT concerning the other blood constituents. This research provides a feasibility  
 64 benchmark between the widely used chemometrics method partial least squares (PLS)  
 65 and the SL-AI method.



**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%)

## 66 2. Materials and Methods

### 67 2.1. Hemogram analysis

68 Dog blood samples from routine clinical practice were collected by qualified per-  
 69 sonnel by standard venipuncture, at the Centro Hospitalar Veterinário do Porto. PLT  
 70 was determined by Beckman-Coulter capillary impedance using Mindray B-2800 vet  
 71 auto-hematology analyzer.

### 72 2.2. Spectroscopy

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

### 78 2.3. Chemometrics

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

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

### 91 3. Results and Discussion

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

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

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

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

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

### 125 4. Conclusions

126 This feasibility study has shown that low intensity, non-dominant, and multi-scale  
127 interferent spectral information is possible to be accessed, by unscrambling information  
128 with the CovM principle included in the SL-AI method. The small variations in the  
129 spectral signal that contain information about PLT cannot be modeled by PLS. SL-AI

**Table 1.** This is a table caption. Tables should be placed in the main text near to the first time they are cited.

Method	SE	LV	R <sup>2</sup>	MAPE(%)	R <sub>Pearson</sub>
PLS	175.99	2	0.068	88.89	0.2613
SL-AI	61.19	1-3	0.7016	24.67	0.8376

130 can unscramble PLT interference information based on the CovM principle, allowing the  
 131 quantification of PLT. Future studies, with more samples, may provide better insights  
 132 on the full potential of the developed POC technology in both veterinary and human  
 133 medicine.

134 **Author Contributions:** Barroso TG, Ribeiro L, and Gregório H: Investigation, methodology,  
 135 validation, writing - review & editing; Santos E: investigation, hardware and firmware; Martins  
 136 RC: conceptualization, software and hardware, funding acquisition, writing - original draft,  
 137 resources and formal analysis, project administration.

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140 **Conflicts of Interest:** The authors declare that they have no known competing financial interests  
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