sysmex | Together for a better healthcare journey

SYSMEX EDUCATIONAL ENHANCEMENT AND DEVELOPMENT | October 2024

SEED Haematology

Recognising special patterns and spurious results in automated blood counting

How to understand the root cause of interferences based on the measurement principles

The use of automated blood counting in clinical laboratories is the essential backbone of patient care and diagnosis. With the adoption of state-of-the-art technologies, the quality and accuracy of the results provided by haematology analysers has improved, thus contributing to a greater reliability and clinical usefulness. The development of various measurement principles has enabled the optimal detection and classification of different types of blood cells, by exploiting their unique morphological characteristics. However, despite these tremendous technological advancements, there are still some rare cases where spurious results can emerge due to certain interfering factors. Most of these interferences can be attributed to specific patient or pre-analytical conditions, whereas some are determined by the technology used [1, 2].

The focus of this SEED article series is to present the potential root causes of some well-characterised special patterns that result in spurious results on the Sysmex 5-part differential haematology analysers, explain which reportable parameters might be affected and highlight aspects of scattergrams and flagging that can support the prompt identification of these patterns.

This article sets the basis for understanding the measurement technologies and channels used in Sysmex haematology analysers and describes the potential reasons why special patterns might occur.

Measurement technologies in automated blood counting

The automated Sysmex 5-part differential analysers employ advanced measurement principles, which take advantage of different cell properties to achieve optimal cell identification. More specifically, the hydrodynamically focussed impedance method is used for the volumetric differentiation and absolute quantification of red blood cells (RBC) and platelets (PLT). The sodium lauryl sulphate (SLS) haemoglobin principle comprises a photometric method that

enables the accurate quantification of the haemoglobin concentration upon generation of a coloured complex (SLS-haemoglobin). Finally, fluorescence flow cytometry technology is used for the identification and quantification of white blood cells (WBC), nucleated red blood cells (NRBC), reticulocytes and platelets based on a three-dimensional analysis, by obtaining information about the physiological and structural properties of the cells (i.e. cell size, intracellular structure and number of nucleic acids and cell organelles) in the form of emitted light.

Each of these principles strongly depends on unique, proprietary reagents, which through interaction with blood cells contribute to their accurate detection and enumeration by exerting their morphological properties and characteristics.

The WNR measurement channel

The WNR channel is part of the complete blood count (CBC) measurement, which comprises the standard analysis profile for every blood sample. The aim of this channel is to determine the total WBC count and differentiate the NRBC and basophils. To achieve optimal cell separation, the blood cells are treated with specifically designed reagents.

For the WBC measurement it is important to lyse RBC as they have the highest concentration in the blood. This is achieved by Lysercell WNR which additionally lyses platelets and perforates the cell membranes of WBC and NRBC. Due to the differences in intracellular pH conditions, this reagent further results in alterations of the cell size. Cells with a basic pH (pH > 7), such as basophils, are not affected by Lysercell WNR and retain their original size. On the other hand, cells with acidic or neutral pH (pH ≤ 7), such as NRBC and other WBC, are shrunk upon treatment with this reagent. This alteration in the cell size enables a better differentiation of the cells detected in the WNR channel.

Fluorocell WNR labels the nucleic acids, as well as several cell organelles, with a specific fluorescent marker. The intensity of the fluorescent signal that the cells emit upon excitation provides valuable information about the metabolic activity and maturation status of the labelled cells, thus contributing further to their accurate differentiation and quantification.

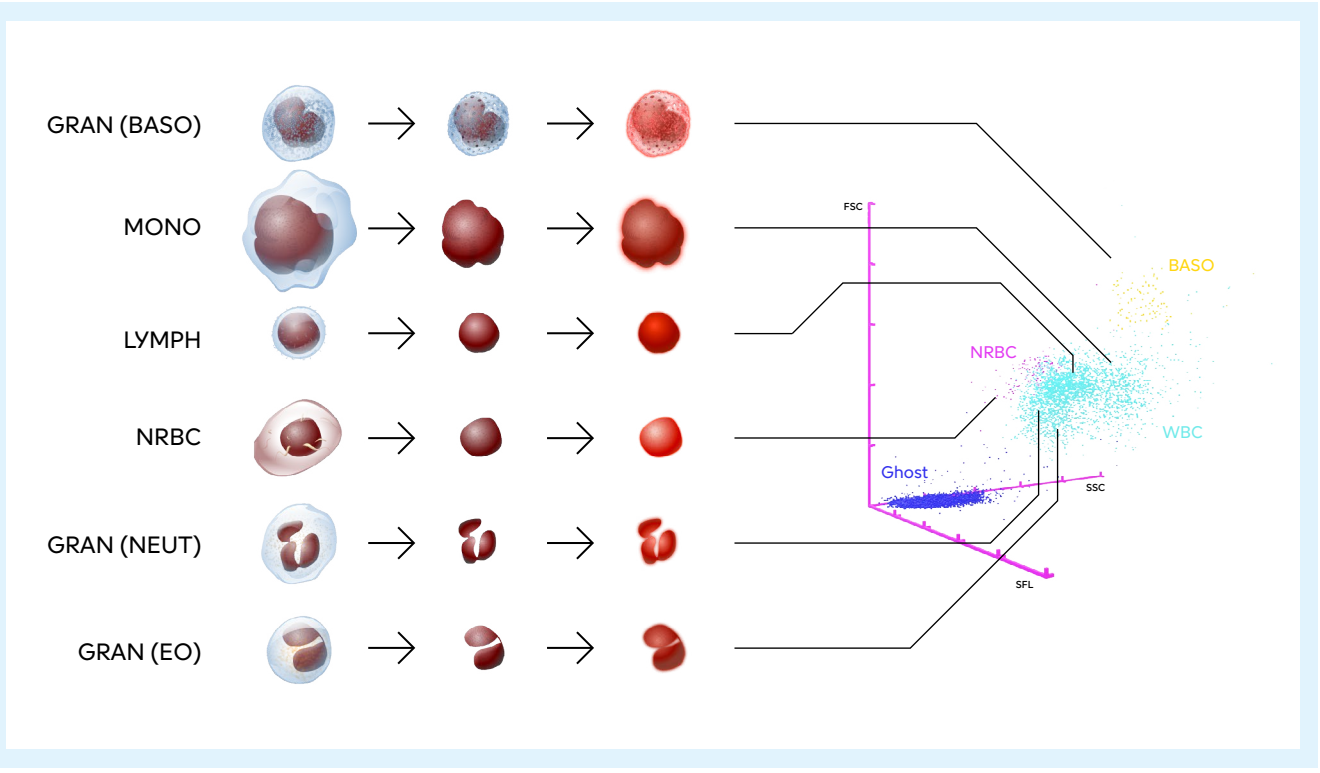

Fig. 1 The WNR reagents exert different effects on the WBC and NRBC, which are related to their individual properties (i.e. cell size (FSC) and nucleic acid content (SFL)). The combination of the information obtained by fluorescence flow cytometry reflects these properties and enables optimal classification and quantification of the detected cells.

The WDF measurement channel

Extended information about the different WBC populations present in a blood sample is provided by the WDF measurement channel. Although the total count is obtained by the WNR channel, when detailed information about the WBC subpopulations is required, the WDF channel is utilised to report the absolute count and percentage of lymphocytes, monocytes, neutrophils and eosinophils. Moreover, if immature granulocytes (IG) are present in the sample, they will be automatically quantified.

In the WDF channel, the precise classification of the cells is attributed to the specific reagents used. The reagents, Lysercell WDF (applicable for the XN-Series and XN-L Series) and Lysercell WDF II (applicable for the XR-Series), also lyse RBC and platelets and perforate the cell membranes of WBC. However, due to a different chemical composition compared to Lysercell WNR, these reagents do not have a direct impact on the size of the WBC, whose main cell characteristics remain largely intact upon the reagent reaction. The only exception is eosinophils. The interaction of the WDF lysing reagents with the acidic granules of eosinophils results in the formation of crystals in these granules, which increases the intracellular complexity of this specific subpopulation. As the WDF channel benefits from differences in intracellular complexity to achieve a correct classification of the WBC subpopulations, this feature of the lysing reagents enhances the channel's performance.

Fluorocell WDF labels the nucleic acids (mostly RNA) and cell organelles (i.e. ribosomes and rough endoplasmic reticulum) with a specific fluorescent marker, thus contributing to an optimal differentiation of the WBC by utilising information that reflects the metabolic activity and maturation status of the labelled cells.

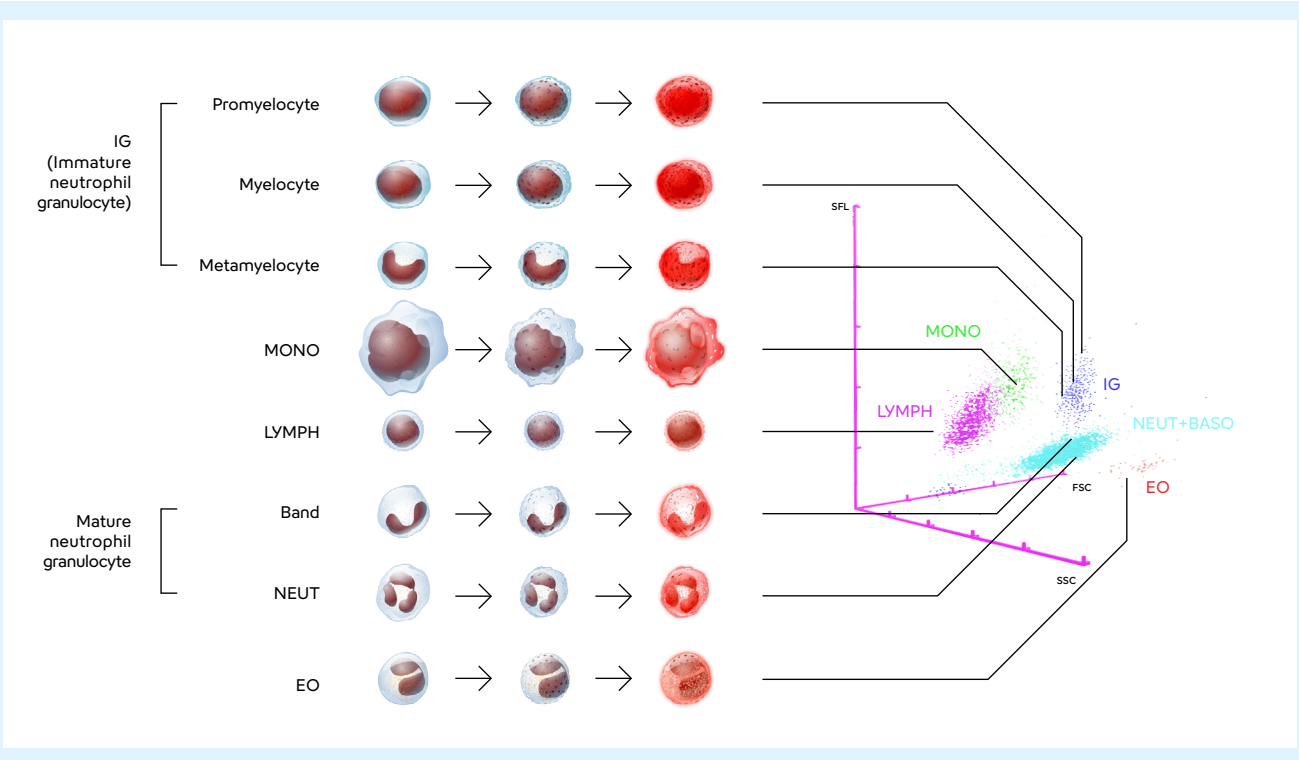

Fig. 2 The WDF reagents exert different effects on the WBC subtypes, which are related to their individual properties (i.e. intracellular complexity (SSC) and nucleic acid content (SFL)). The combination of the information obtained by fluorescence flow cytometry reflects these properties and enables optimal classification and quantification of the detected cells.

The WPC measurement channel

In case the presence of abnormal or potentially reactive WBC is suspected, additional relevant information can be provided by the WPC channel. Although the first level of awareness can be raised by a generic flag triggered in the WDF channel, the WPC channel can reveal further details about the haematopoietic origin of the abnormal cells and trigger explicit flags with a higher specificity.

As for the other two channels, specific reagents are used to differentiate between suspected immature or abnormal WBC and mature WBC. In general, the cell membranes of immature cells contain fewer lipids than those of mature cells. Consequently, the reagent Lysercell WPC, which apart from lysing RBC and platelets also perforates the membranes of WBC, exerts a stronger effect on mature cells, which are more efficiently permeated than immature cells. Due to the different degree of cell membrane perforation, the amount of Fluorocell WPC that labels the nucleic acids (mainly DNA) of mature and immature cells varies, hence contributing to a better separation.

Similarly, activated reactive cells can be easily permeated by Lysercell WPC and exert a high fluorescence intensity, which reflects their increased metabolic activity.

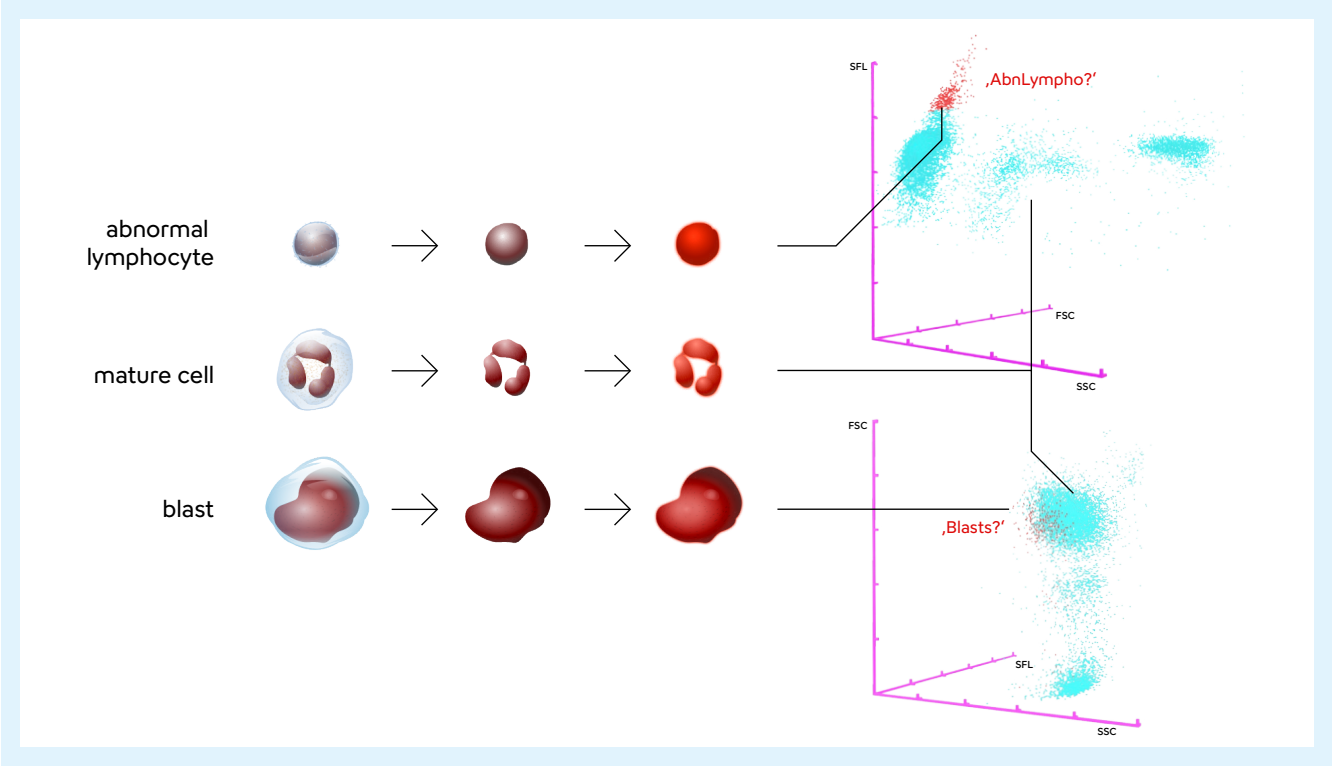

Fig. 3 The WPC reagents exert different effects on the immature, mature and activated WBC, which are related to their individual properties (i.e. intracellular complexity (SSC), nucleic acid content (SFL) and/or cell size (FSC)). The combination of the information obtained by fluorescence flow cytometry reflects these properties and enables optimal differentiation of the detected cells. The images above are representative examples showing where these cells may appear in the WPC scattergrams. However, the exact position of immature cells depends on the underlying pathology, and variations of these patterns can occur.

The RET measurement channel

Although RBC are primarily measured by the hydrodynamically focussed impedance method, which is based on volumetric cell differentiation, the RET channel can be used to provide additional information about reticulocytes and their different maturation levels.

This channel uses fluorescence flow cytometry to accurately quantify reticulocytes. The reagent Cellpack DFL does not exert any lysing properties, but rather perforates the cell membranes of RBC and reticulocytes. The reagent Fluorocell RET labels the remaining RNA in the reticulocytes' cytoplasm with a specific fluorescent marker. Since in the course of erythropoiesis, reticulocytes present in different maturation levels, which correspond to the gradual loss of nucleic acid content, the fluorescence intensity of the detected cells is used to classify them into high-, medium- and low-fluorescence reticulocytes (HFR, MFR, LFR) and mature RBC.

As no lysing is applied on any blood cell type, the RET channel can also be used for the quantification of platelets (PLT-O) in the sample. This is particularly helpful in case the platelets cannot be easily differentiated from the RBC by volumetric methods, such as in samples presenting microcytes, fragmented RBC or giant platelets.

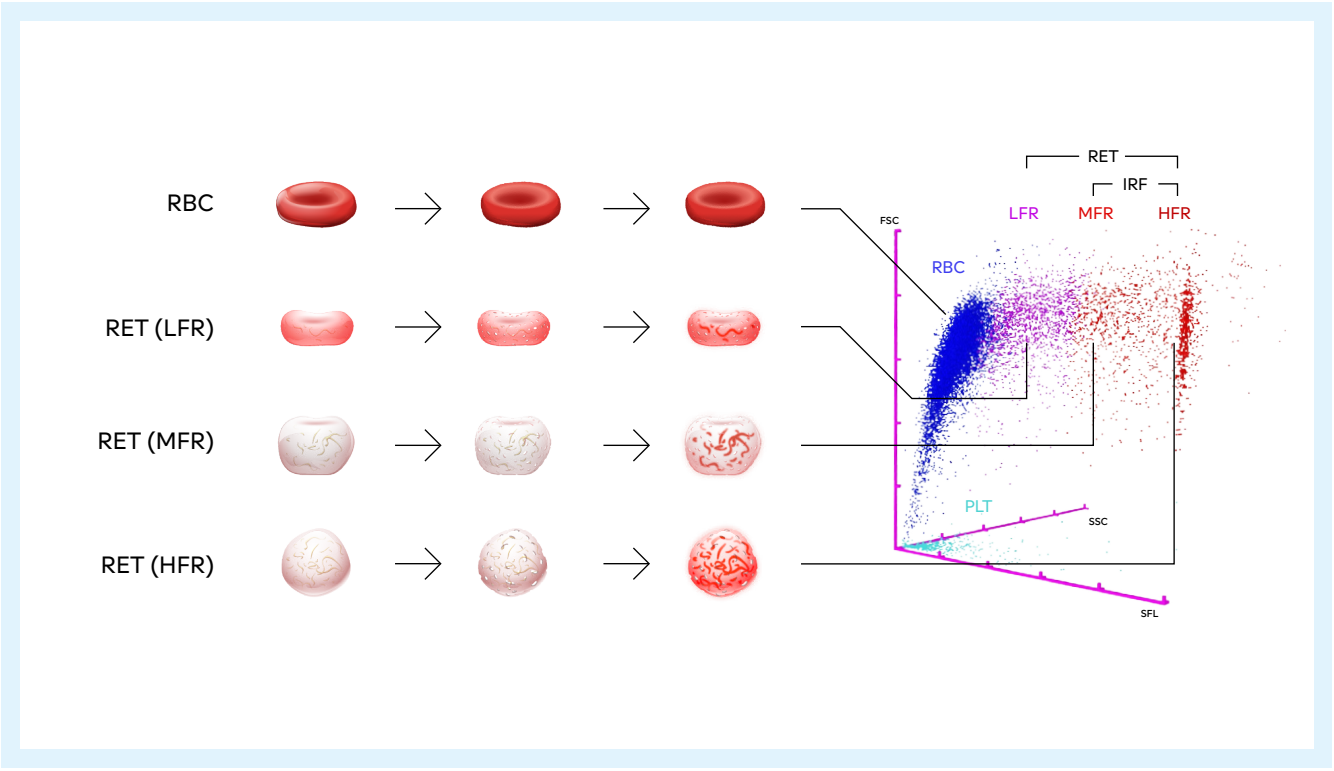

Fig. 4 RET channel is used for the quantification of reticulocytes of different maturation levels. The combination of the information obtained by fluorescence flow cytometry (i.e. nucleic acid content (SFL) and cell size (FSC)) reflects the different properties of each cell population and enables their optimal classification. Moreover, mature RBC and platelets can be detected and quantified.

The PLT-F measurement channel

The PLT-F measurement channel was designed to obtain a highly precise and reliable platelet count. Due to its larger counting volume and use of fluorescence compared to impedance measurement, this channel can more accurately quantify the platelet count of severely thrombocytopenic samples [3] and overcome interferences that affect correct platelet classification based on their size [4, 5, 6].

Similarly to the RET channel, the reagent Cellpack DFL perforates the cell membranes of the platelets, and the reagent Fluorocell PLT labels the RNA present in the cytoplasm with a specific fluorescent marker. As immature or reticulated platelets contain a higher amount of RNA and thus will exert a higher fluorescence signal, this method allows for optimal separation between immature and mature platelets and accurate quantification of each population compared to the reference method [3, 4, 5].

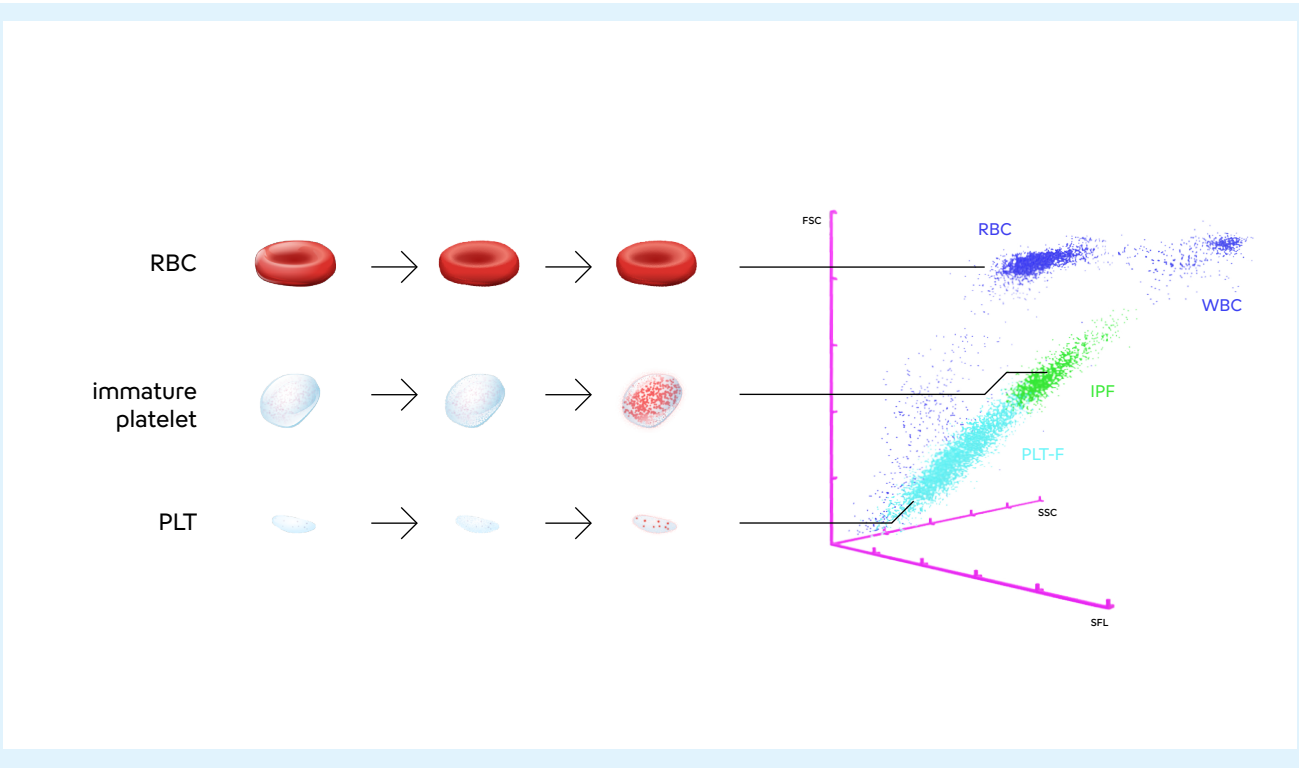

Fig. 5 The PLT-F channel can precisely determine the platelet count by employing the fluorescence flow cytometry method. The information obtained (i.e. nucleic acid content (SFL) and cell size (FSC)) reflects the different maturation stages of platelets, thus enabling optimal classification and accurate quantification of immature and mature platelets.

Spurious results due to interfering factors

As mentioned before, despite the technological improvements in haematology analyser hardware and software, erroneous results might be generated in rare cases due to interfering factors. These interferences usually result in abnormal sample characteristics, and the most common causes can be broadly classified in two groups:

- underlying pathologies and
- pre-analytical factors [1, 2].

It is well known that cell properties might be altered due to clinical causes. Various pathological conditions or therapeutic regimes can affect the size, structure, maturation level or metabolic activity of a cell or may impact the proper interaction with the reagents used on the automated haematology analysers. Similarly, pre-analytical factors, such as incorrect sample volume, excessive mixing, incorrect blood draw, anticoagulants or nutritional factors can also affect the cell properties, as well as the reagent reactions. Some of the most common observations made when such interferences occur are cell aggregation, cell fragmentation, cell inclusions, resistance to lysis or the presence of non-cellular particles that can be incorrectly perceived by the analysers as cells [1, 2].

Depending on the type of interference, various haematological parameters may be affected, and distinct abnormal patterns may appear on the histograms and/or scattergrams. Over the past years, Sysmex has made continuous improvements in identifying and characterising common atypical patterns, as well as their impact on analysis results. The outcome of this effort is that these interferences are listed in the Instructions for Use (IFU) of each haematology analyser and specific flag messages can be generated to alert the operators in case of possible abnormal findings.

Examples of these special patterns and spurious results affecting various channels are described in the other articles of this SEED article series.

Known causes of spurious results in automated blood counting [1, 2]

Pre-analytical factors	Underlying pathologies
■ Cell aggregation	■ Cell aggregation
■ Fragmented cells	■ Fragmented cells
■ Lipaemia	■ Cold agglutination
	■ Cryoglobulins
	■ Cell inclusions
	■ Microorganisms
	■ Lyse-resistant cells

Concluding remarks

The widespread use of automated blood counting has set the pace for continuous improvement in the accuracy of the results obtained by haematology analysers. Despite the tremendous efforts towards better analytical performance, various pre-analytical or analytical interfering factors can contribute to the generation of spurious results. The improvement in blood cell analysis, in combination with awareness around limitations of the current measurement principles has brought attention to the careful examination of related histograms and scattergrams for identifying potential abnormalities. Well-characterised atypical patterns and related flags can alert the user of suspicious results and in many cases be suggestive of the cause of the pattern. This can be of immense support for the users, who can more easily decide on the next steps that need to be taken to ensure accurate results and a reliable diagnosis.

References

[1] Zandecki M *et al.* (2007): Spurious counts and spurious results on haematology analysers: a review. Part I: platelets. Int J Lab Hematol; 29(1): 4–20.

[2] Zandecki M *et al.* (2007): Spurious counts and spurious results on haematology analysers: a review. Part II: white blood cells, red blood cells, haemoglobin, red cell indices and reticulocytes. Int J Lab Hematol; 29(1): 21–41.

[3] Schoorl M *et al.* (2013): New fluorescent method (PLT-F) on Sysmex XN2000 hematology analyzer achieved higher accuracy in low platelet counting. Am J Clin Pathol;140:495.

[4] Tanaka Y *et al.* (2014): Performance evaluation of platelet counting by novel fluorescent dye staining in the XN-Series automated hematology analyzers. J Clin Lab Anal; 28(5):341-8.

[5] Wada A *et al.* (2015): Accuracy of the New Platelet Count System (PLT-F) Depends on the Staining Property of Its Reagents. PLOS ONE; 10(10).

[6] Tantanate C (2015): Spuriously high platelet counts by various automated hematology analysers in a patient with disseminated intravascular coagulation. Clin Chem Lab Med; 53(10):e257-9.