XN Training Manual (Part I)

XN Series Training Manual (Part I)

Revision 10: February 2019

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Table of Contents

PURPOSE AND SCOPE OF THE MANUAL 5

SECTION 1: INTRODUCTION 6

Introduction 7

Instrument overview 7

Reagent system 8

Reagent summary 10

SECTION 2: ANALYSIS PRINCIPLES 11

Analysis Principles 12

Fluorescent Flow Cytometry 13

WNR Channel (WBC analysis) 14

Differential Analysis (WDF Channel) 16

Adaptive Cluster Analysis (ACAS) 19

Sysmex Adaptive Flagging Algorithm based on Shape- recognition (SAFLAS) 20

White Precursor Cells (WPC Channel) this channel is only applicable for XN-20 module 21

Reticulocyte (RET Channel) 24

Fluorescent Optical PLT (PLT-O) 27

Fluorescent PLT channel (PLT-F) 28

Sheath Flow Direct Current (DC) Detection method 30

SLS-Haemoglobin Method 33

XN Flagging 35

Limitation of Flagging 36

Interpretive (IP) Message 38

Q-Flag 39

SECTION 3: BODY FLUID, HPC AND HSA 41

Body Fluid analysis 42

HPC analysis (only applicable for XN-20 module with activated HPC license) 43

hSA analysis (only applicable for XN module with activated BF and RET licenses) 44

SECTION 4: PRACTICAL ASSESSMENTS 45

Knowledge Review 46

Purpose and Scope of the Manual

The XN training manual has been prepared to provide a basic understanding of the XN analysers, the technologies and methodologies employed by the systems, general operations and maintenance. It is NOT intended to replace the Sysmex XN Instruction For Use or Operator manual.

New operators are advised to read the Operators and Instructions For Use manuals before use, and to take particular notice of all Warnings and Safety Information notices.

Section 1: Introduction

Introduction

Sysmex XN-series are the next generation of full blood count analysers for in vitro diagnostic use in Haematology laboratories.

XN instrument enables the analysis of tangible components of blood and body fluid by means of electrical impedance, laser light scattering, and dye bonding.

Models available are the XN-10 and XN-20. In addition, XN-20 has additional WPC (White Precursor cells) Channel used for differentiation of Blasts and Abnormal Lymphocytes. Data is stored and displayed on the Information Processing Unit (IPU).

Throughput:

XN-10: maximum of 100 (CBC+ DIFF+ NRBC), samples per hour

XN-20: maximum of 100 (CBC+ DIFF+ NRBC), samples per hour

Instrument overview

The XN series haematology system consists of the following:

- Analyser (XN-10/XN-20)
- Sampler section (SA-10/SA-01/SA-20/SA-30, Transportation units (CV-50/55, CV-60/65, CV-70, and TU)
- IPU
- Pneumatic unit
- Printer
- SP-10
- Additional components (The components and options may be sold as individual units)
    - Wagons

Reagent system

CELLPACK DCL

- Diluent used, for measuring the numbers and size of RBC and PLT (hydro dynamic focusing-DC detection).
- By addition of lyse reagent it is also used for HGB concentration determination.
- CELLPACK DCL is also used as sheath fluid for FCM detector.

CELLPACK DST

- Concentrated diluent used, for measuring the numbers and size of RBC and PLT (hydro dynamic focusing-DC detection).
- By addition of lyse reagent it is also used for HGB concentration determination.
- CELLPACK DST is also used as sheath fluid for FCM detector.
- This reagent is to be used by connecting to reagent preparation device specified by RDA

SULFOLYSER

- Used for automated determination of HGB concentration in Whole Blood.

CELLPACK DFL

- Diluent used in combination with Fluorocell RET for RETIC analysis.
- Diluent used in combination with Fluorocell PLT for PLT-F analysis

LYSERCELL WNR

- Lysing agent used to haemolyse RBC for WNR channel.
- This reagent Combined with Fluorocell WNR determines WBC (non-basophil), basophils and NRC population.

LYSERCELL WDF

- Lysing agent used to haemolyse RBC for WDF channel.
- This reagent combined with the Fluorocell WDF is used to determine 4 part DIFF (Neutrophils, Lymphocytes, Monocytes and Eosinophil's)

LYSERCELL WPC (on XN-20 system only)

- lysing agent used to haemolyse RBC for WPC channel.
- This reagent combined with the Fluorocell WPC to determine presence of abnormal or immature WBC cells (separation of Abnormal Lymphocyte and Blast cell population)

FLUOROCELL WNR

- Staining reagent used in WNR channel.
- This reagent will stain NRBC, WBC (no-basophil) and Basophil cells in the WNR channel.

FLUOROCELL WDF

- Staining reagent used in WDF channel. This reagent will stain leukocytes (Neutrophil, Lymphocyte, Monocyte and Eosinophil) for 4 part DIFF in the WDF channel.

FLUOROCELL RET

- Staining reagent used in RET channel.
- This reagent will stain RET and PLT cells in the RET channel for determination of RET cells and O-PLT.

FLUOROCELL PLT

- Staining reagent used in Fluorocell-PLT channel.
- This reagent will stain PLT cells in the Fluorescent PLT channel for determination of Fluorescent PLT.

FLUOROCELL WPC (on XN-20 system only)

- Staining reagent used in WPC channel.
- This reagent will stain WBC separating immature cells (Blast) from abnormal cells (Abnormal Lymphocytes).

CELLCLEAN-AUTO

- Cleaning fluid, removes lysing reagent, cellular residue and blood proteins from hydraulic system, detectors and aspiration tubes.

Reagent summary

CATALOUGE NUMBER	REAGENT	VOLUME	CYCLE PER PACK	SHELF LIFE AFTER OPENING
06977197001	CELLPACK DCL	20L	380-750	60 DAYS
06977189001	CELLPACK DCL	10L	190-370	60 DAYS
06510205001	CELLPACK DFL	2 x 1.5L	2000	60 DAYS
06709826001	CELLPACK DST Concentrate*	4L	3000	60 DAYS
06510191001	CELLPACK DST Concentrate*	10L	7500	60 DAYS
06510183001	CELLPACK DST Concentrate*	20L	15000	60 DAYS
06646409001	SULFOLYSER	2 x 1.5L	6000	60 DAYS
08128553001	LYSERCELL WNR	1 x 5L	3300	60 DAYS
06510248001	FLUOROCELL WNR	2 x 82mL	8000	90 DAYS
08128537001	LYSERCELL WDF	1 x 5L	3300	90 DAYS
06510256001	FLUOROCELL WDF	2 x 42mL	4000	90 DAYS
06510230001	LYSERCELL WPC	2 x 1.5L	2000	90 DAYS
06510264001	FLUOROCELL WPC	2 x 12 mL	1000	90 DAYS
06510272001	FLUOROCELL RET	2 x 12 mL	1000	90 DAYS
06510299001	FLUOROCELL PLT	2 x 12 mL	1000	90 DAYS
06975445001	CELLCLEAN AUTO	20 x 4mL	20	As indicated on packaging

Table 1- Reagent Summary

SECTION 2: ANALYSIS PRINCIPLES

Analysis Principles

Refer to Chapter 15 of the Instruction for Use Manual for further detail.

The XN employs 3 primary analysis principles.

- **Fluorescent Flow Cytometry -** using a semiconductor laser at 633 nM.
- **Hydro Dynamic Focusing (DC detection)-** RBC/PLT analysis
- **SLS Haemoglobin method** – cyanide free HGB analysis

Fluorescent Flow Cytometry

A Semi-Conductor Laser is used to analyse the physiological and chemical characteristics of cells and other biological particles. A blood sample is aspirated and measured, diluted to the specified ratio, and stained and then fed into the flow cells.
Hydro Dynamic Focusing mechanism improves cell count accuracy and reproducibility

A semiconductor laser beam is emitted to the blood cells passing through the flow cell.

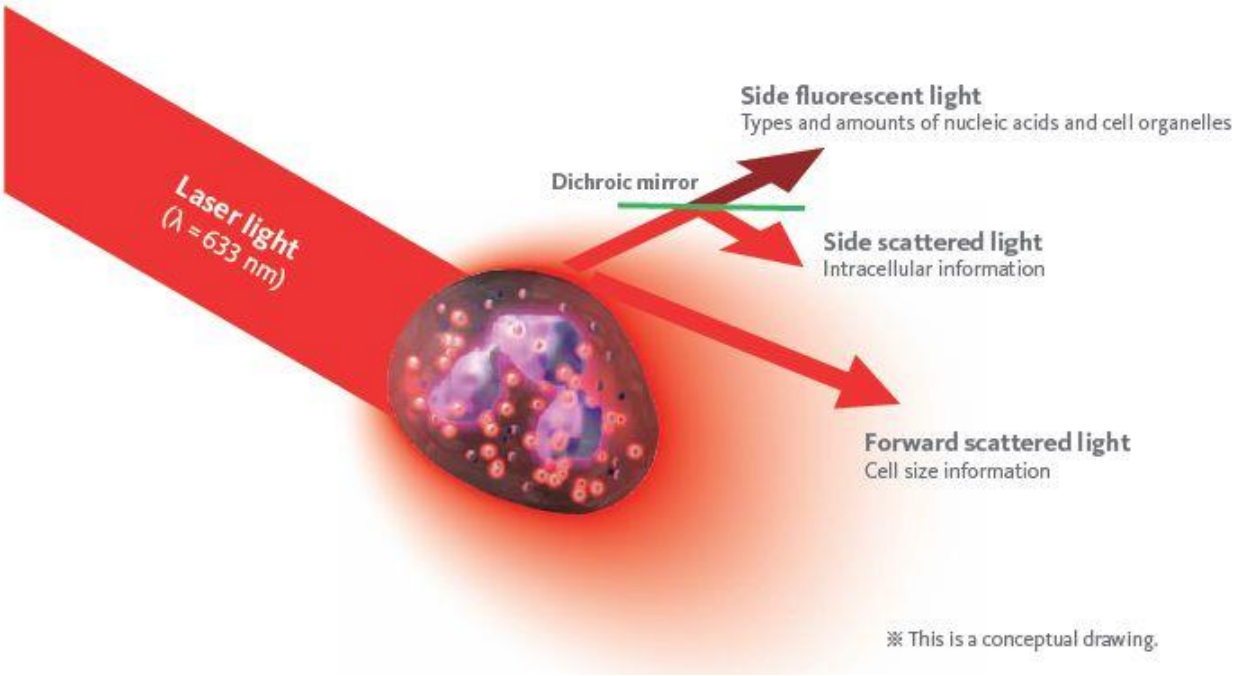


Figure 1- Laser Flow Cytometry

- **Forward Scattered Light** indicates cell volume.
- **Side Scattered Light** indicates intercellular information such as granularity and lobularity.
- **Side Scattered Fluorescent Light** indicates type and amount of nucleic acids and cell organelles including RNA / DNA content of the cell.

XN system employs Flow Cytometry technology in following channel:

WNR, WDF, WPC, RET and PLT-F

WDF channel engages further technology to identify each WBC cluster displaying final results on the WDF Scattergram. Each cell that passes through the laser generates a unique signature. Cell scatter properties are classified by the Sysmex Adaptive Cluster Analysis (ACAS) as well as Sysmex Adaptive Flagging Algorithm based on Shape-recognition (SAFLAS). These signals are converted into the Scattergram information displayed on the XN IPU.

WNR Channel (WBC analysis)

The WNR channel is used to count the white blood cells and nucleated red blood cells.
By means of flow cytometry method, a two-dimensional scattergram is plotted, with the X-axis representing the intensity of the lateral fluorescent light (SFL), and the Y-axis representing the intensity of the forward scattered light (FSC).

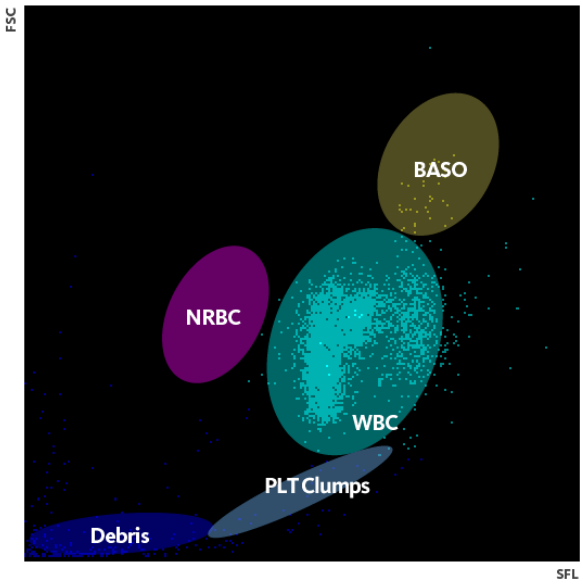


Figure 2- WNR Scattergram

This scattergram displays groups of:

- Nucleated red blood cells,
- Basophil
- Non-basophil white blood cells
- Debris (haemolysed red blood cells and platelets).

Following parameters are numerated:

- WBC
- NRBC#
- NRBC%,
- BASO#
- BASO%.

Flags generated from this channel include:

- PLT Clumps?
- NRBC Present
- WBC Abnormal Scattergram

Whole Blood Analysis – WNR

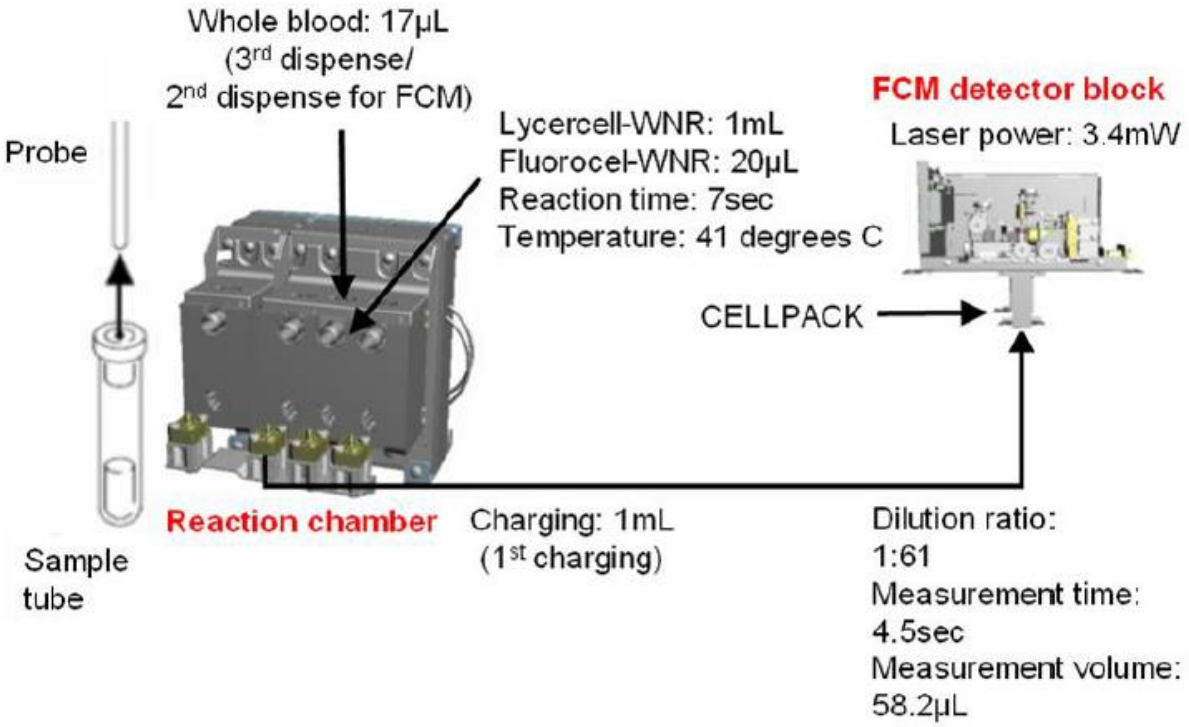


Figure 3- WB analysis flow WNR Channel

Differential Analysis (WDF Channel)

The WDF channel is used for classifying white blood cells. By means of flow cytometry method, a two-dimensional scattergram is plotted, with the X-axis representing the intensity of the side scattered light (SSC) and the Y-axis representing the intensity of the lateral fluorescent light (SFL).

This scattergram displays Lymphocytes, Monocytes, Eosinophils, Basophils + Neutrophils, and Debris.

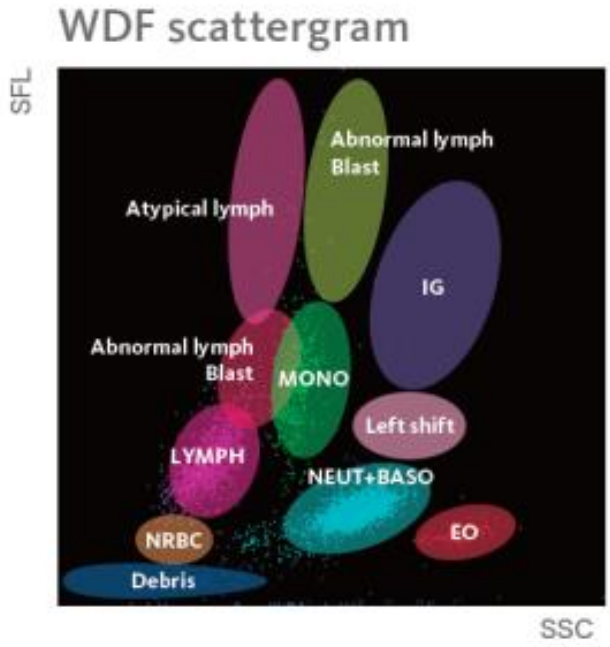


Figure 6- WDF Scattergram

Using reagents Lysercell WDF and Fluorocell WDF channel will provide classification and numeration of following Parameters and analytical parameters:

Parameters:

- NEUT# and NEUT%
- LYMPH# and LYMPH%
- MONO# and MONO%
- EO# and EO%
- IG# and IG%
- WBC-BF

Analytical Parameters:

- MN# and MN%
- PMN# and PMN%
- TC-BF#

Flagging generated from WDF channel includes:

- Atypical lymph?
- Blast/Abn Lymph?
- IG present, PLT Clumps?
- WBC Abnormal Scattergram

Whole Blood Analysis – WDF

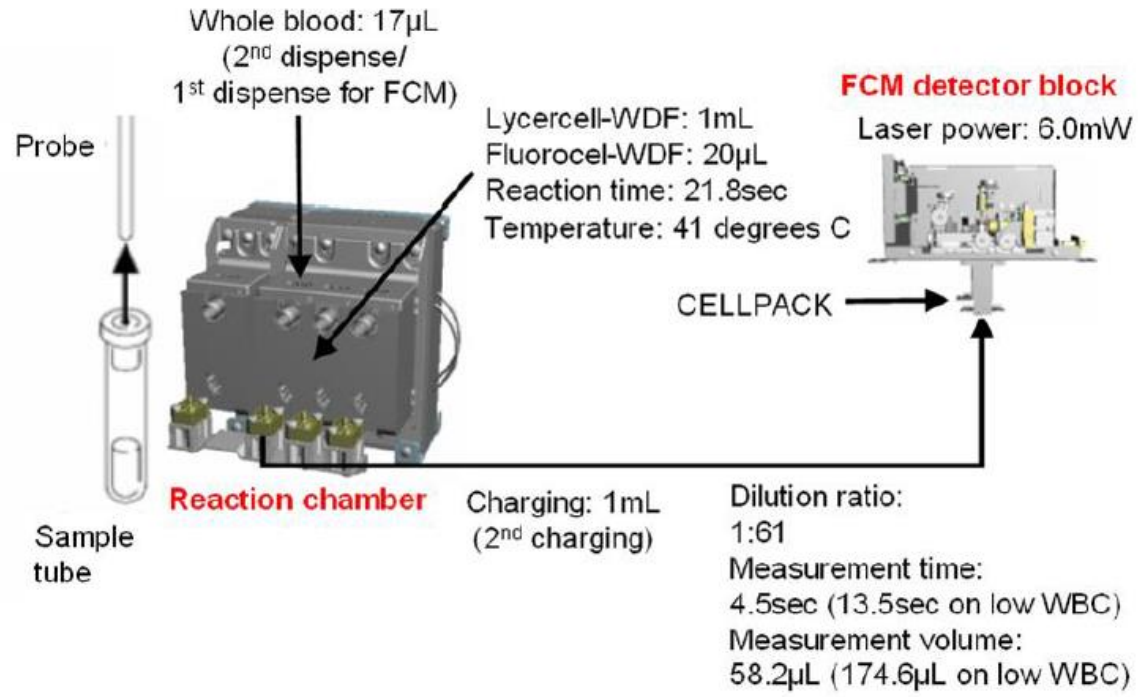


Figure 7- WB analysis flow WDF

Body Fluid Analysis – WDF

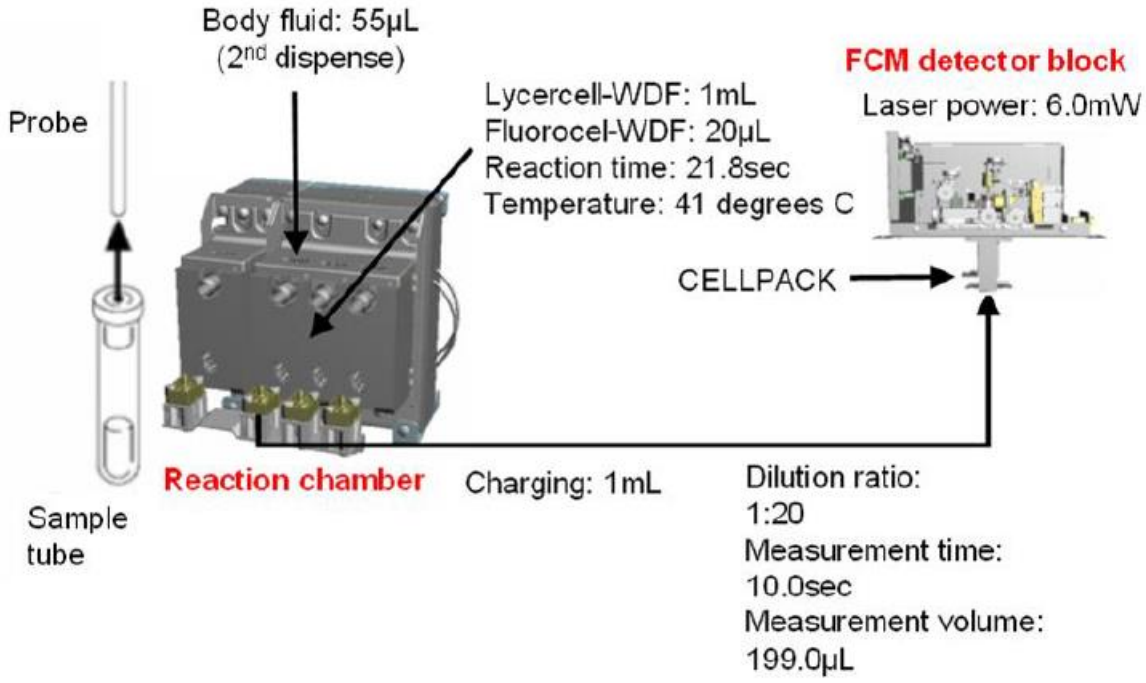


Figure 8- BF analysis flow WDF

Adaptive Cluster Analysis (ACAS)

Classification of cell populations in WDF Channel (Differential channel) is based upon competitive learning algorithms. Each cell produces a unique “cell signature” or position on the scattergram, as it passes through the laser beam.

The default center of the cluster is an empirically defined value, but when the competitive learning algorithm (ACAS based on Mahalanobis distance between populations) is applied, individual variations in cell characteristics can be defined.

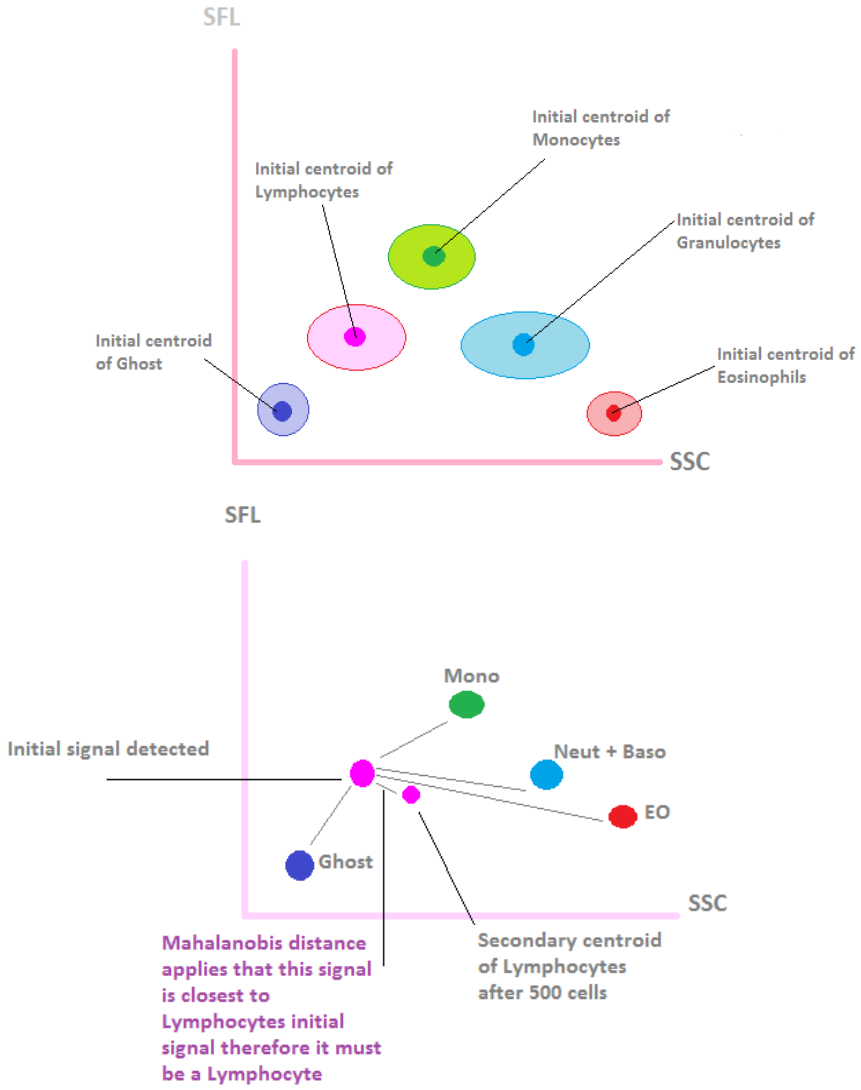


Figure 4- Adaptive Cluster Analysis

If the analyser cannot clearly separate the cell population clusters, the clusters are GREYED OUT, and the associated flagging messages will be displayed on the IPU.

This method of “adaptive” cluster analysis allows an optimal adaptation to individual biological differences between samples even with highly abnormal patterns.

Sysmex Adaptive Flagging Algorithm based on Shape- recognition (SAFLAS)

In addition to ACAS, SAFLAS algorithm is used in classification of abnormal Lymphocyte and Monocyte population in WDF (Differential channel).

Using SAFLAS algorithm, XN will look at individual cell characteristics such as:

Cluster position, Cluster area, Cluster shape and size, Cluster angle and Center of gravity before classifying them in to their respective clusters.

Normal **Abnormal (Blast?)** **Abnormal (Abn Lymphocyte)**

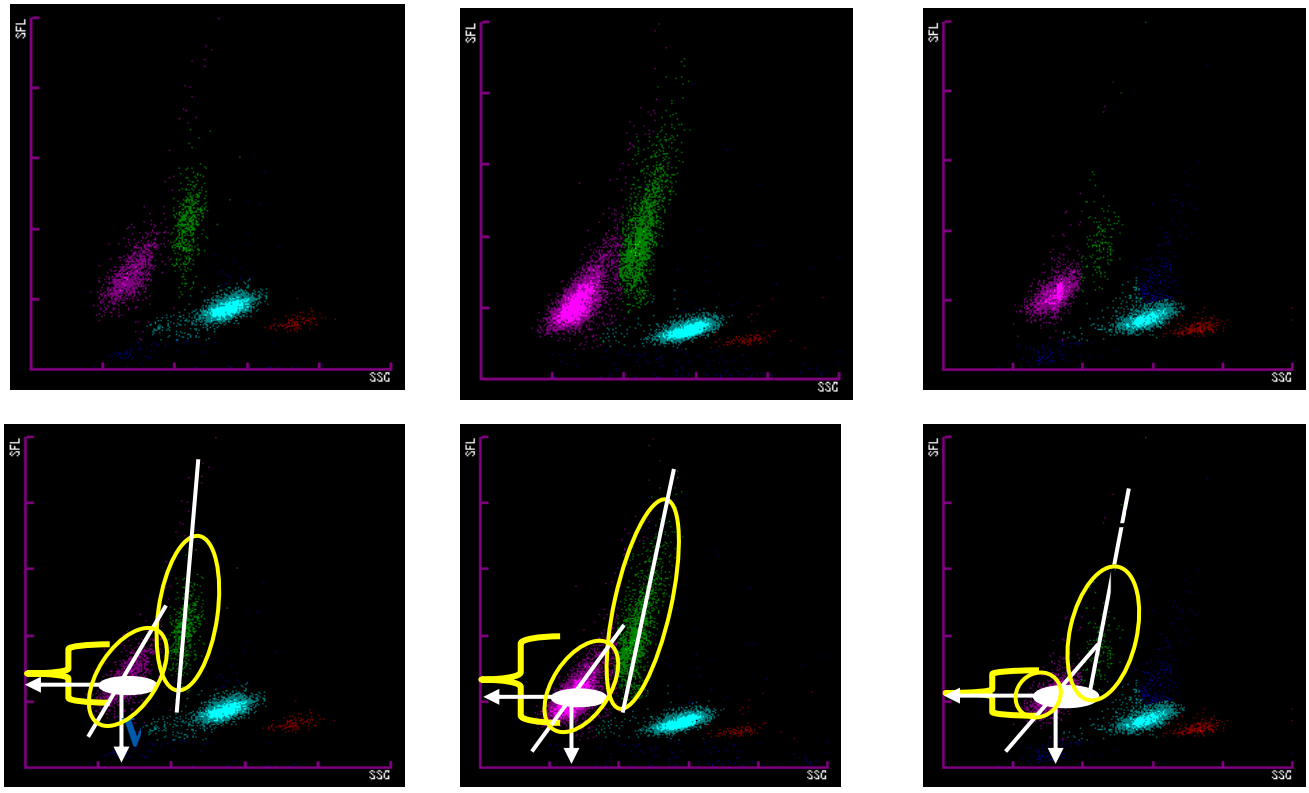


Figure 5- SAFLAS Scattergram

Cluster position: ⬯
Cluster area: {
Cluster shape and size: ⬯
Cluster angle: /
Center of gravity: ↑ and white dot

White Precursor Cells (WPC Channel) this channel is only applicable for XN-20 module

The WPC channel is used for detection of immature cells such as Blasts, and Abnormal Lymphocytes.

By means of Fluorescent flow cytometry method using a semiconductor laser, a two-dimensional scattergram is plotted, with the X-axis representing the intensity of the side scattered light (SSC) and the Y-axis representing the intensity of the lateral fluorescent light (SFL).

Using reagents LYSERCELL WPC and FLUOROCELL WPC, WPC channel will aid in Detection for immature cells (Blast or Abnormal Lymph).

Flagging generated from WPC channel includes: Abnormal lymph?, Blasts

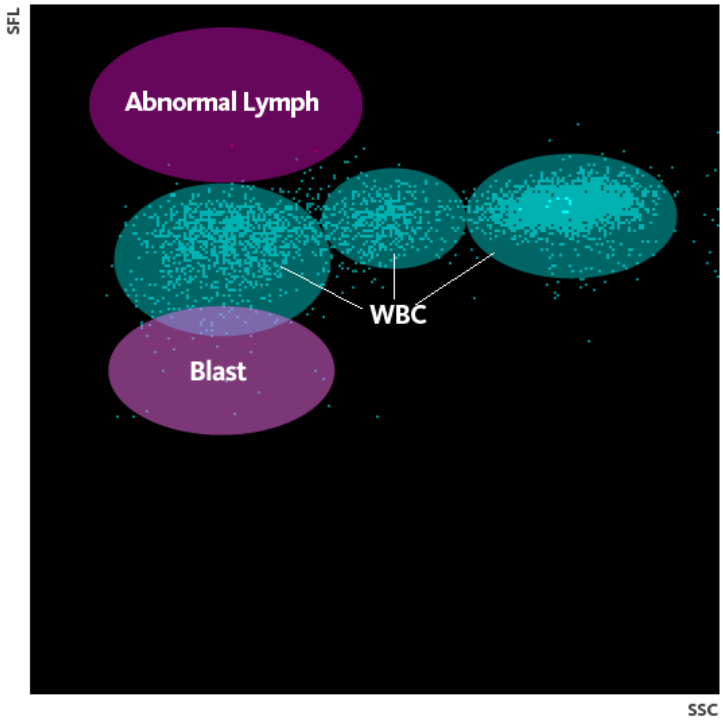


Figure 9- WPC Scattergram

Scattergram figure 9; differentiates immature cells based on Abn Lympho strong SFL when compared to the Blast cells.

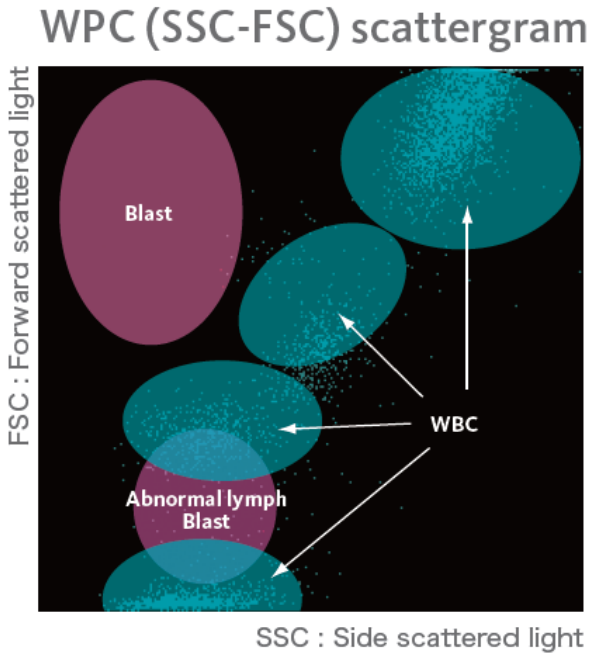


Figure 10- WPC Scattergram

Scattergram figure 10; differentiates immature cells based on Blast cells larger cell volume when compared to Abn Lympho cells.

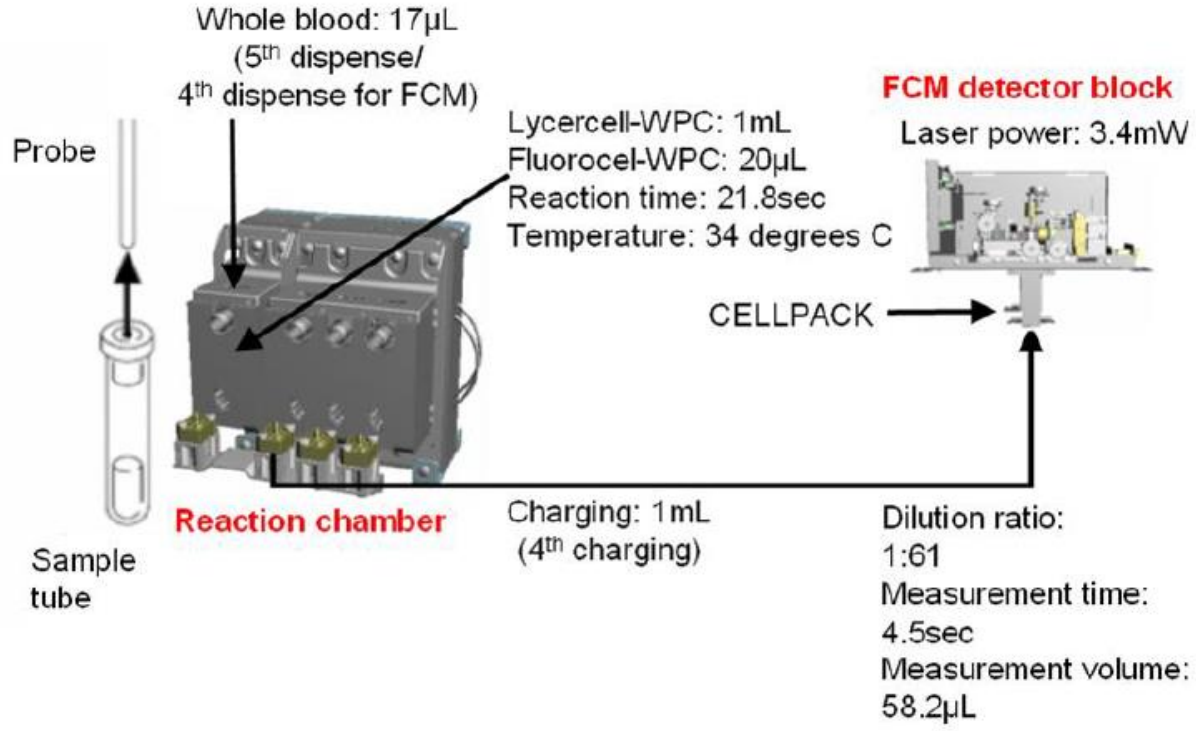


Figure 11- WBC analysis WPC Channel

Reticulocyte (RET Channel)

By means of Fluorescent flow cytometry, a two-dimensional scattergram is generated, with the X-axis representing the intensity of the lateral fluorescent light (SFL), and the Y-axis representing the intensity of the forward scattered light (FSC).

Using reagents CELLPACK DFL and FLUOROCELL RET, the Retic channel classifies reticulocytes and their sub-populations. The fluorescent optical platelet count PLT-O is also derived from this channel.

Flagging generated from RET channel includes: RET Abnormal scattergram, Fragments?

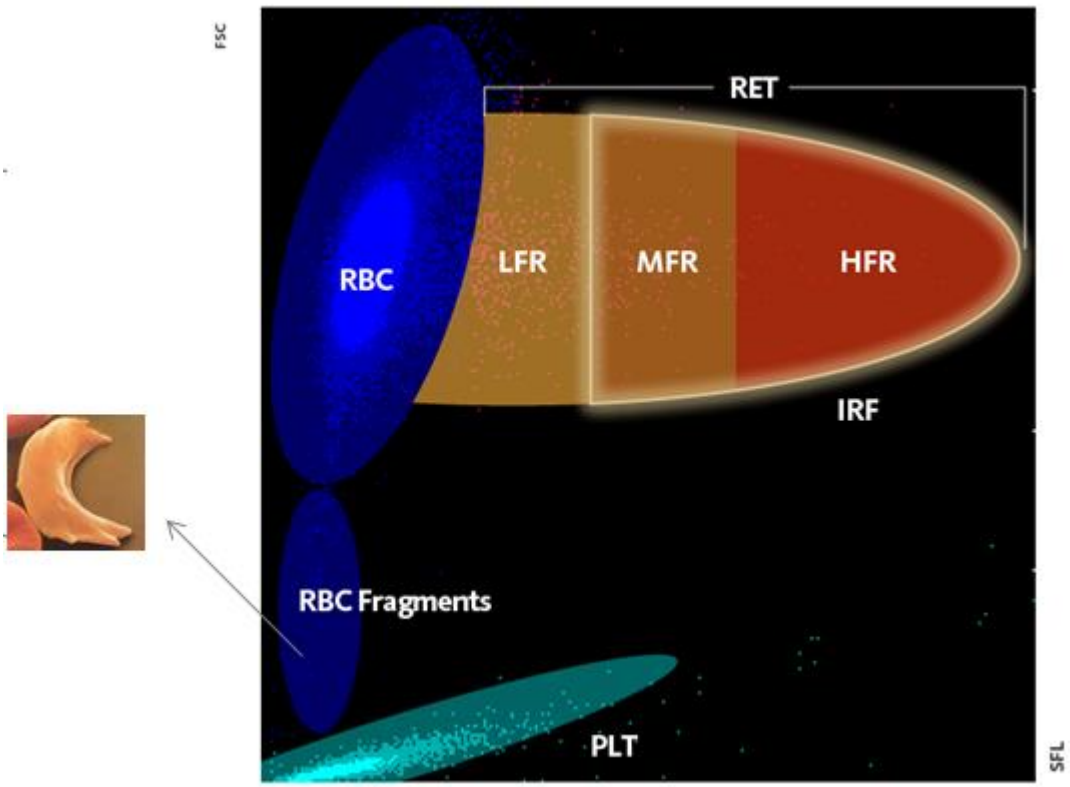


Figure 12- RET Scattergram

The scattergram is divided into three RET zones based on the intensity of the fluorescent light, and the ratio of the reticulocytes in each zone to the total number of reticulocytes is calculated.

Reticulocyte Ratio:

$$\text{RET \%} = \frac{\text{Particle count in reticulocyte zone}}{\text{Particle count in mature RBC zone + Particle count in reticulocyte zone}} \times 1000$$

Reticulocyte Count:

$$RET\# = \frac{RET\% \times RBC}{100}$$

Low Fluorescence Ratio:

LFR = 1000 - HFR – MFR

Middle Fluorescence Ratio:

$$MFR = \frac{\text{Particle count in MFR zone}}{\text{Particle Count in reticulocyte zone}} \times 1000$$

High Fluorescence Ratio:

$$HFR = \frac{\text{Particle count in HFR zone}}{\text{Particle count in reticulocyte}} \times 1000$$

Immature Reticulocyte Fraction:

IRF = MFR + HFR
LFR: Low Fluorescence Ratio
MFR: Middle Fluorescence Ratio
HFR: High Fluorescence Ratio
IRF: Immature Reticulocyte Fraction
RET-He (Reticulocyte Haemoglobin equivalent)

Whole Blood Analysis – RET

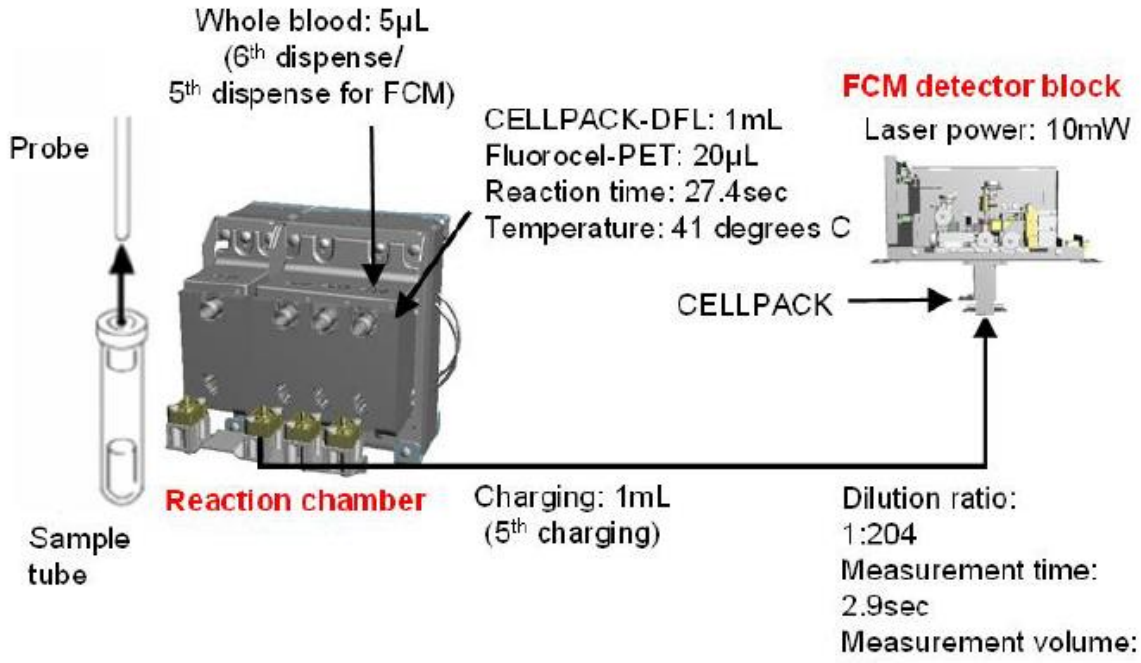


Figure 13- Whole blood Analysis RET

Fluorescent Optical PLT (PLT-O)

The Reticulocyte scattergram analysis is used to provide the Fluorescent Optical Platelet count, which is displayed on the XN-IPU screen. The PLT-O count is clinically useful when the patient's sample contains RBC fragments.

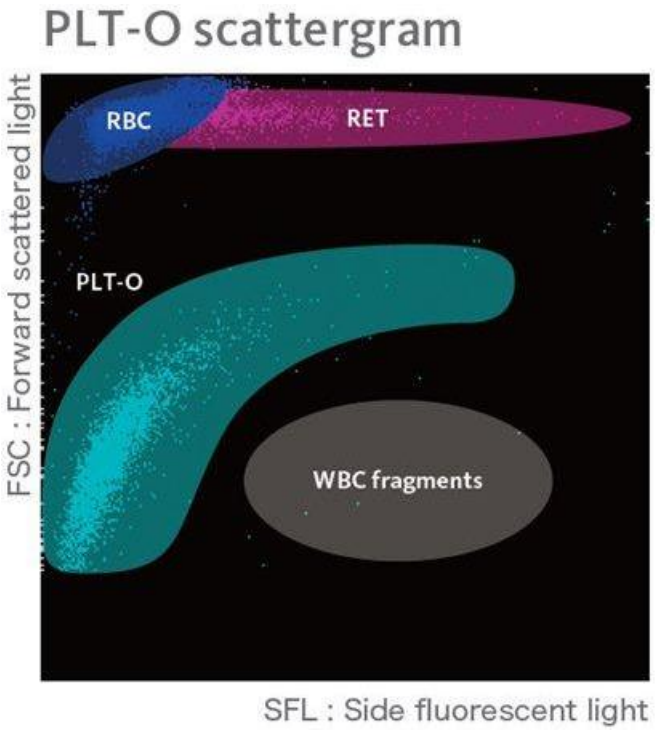


Figure 14- PLT-O Scattergram

Fluorescent PLT channel (PLT-F)

The PLT-F channel is used as a third option for measuring platelets, especially intended for use at low platelet levels.
By means of fluorescent flow cytometry using a semiconductor laser, a two-dimensional scattergram is plotted, with the X-axis representing the intensity of the lateral fluorescent light (SFL), and the Y-axis representing the intensity of the forward scattered light (FSC).

Using reagents CELLPACK DFL and FLUOROCELL PLT, PLT-F channel classifies PLT and Immature Platelet Fraction populations.

The IPF is obtained as a ratio of platelet count in the area with strong fluorescent light intensity in the PLT-F scattergram (IPF zone), to the total platelet count.

IPF (Immature Platelet Fraction):

$$IPF = \frac{\text{Particle count in IPF zone}}{\text{Particle count in the platelet zone}} \times 1000$$

Flagging generated from PLT-F channel includes: PLT Abnormal scattergram, PLT Clumps?

The fluorescent dye (Oxzine dye) in PLT-F channel is different from RET channel and Reticulocyte is not dyed by PLT-F staining solution. PLT- F channel option will also extended the PLT count cycle, making it possible to extend the count by 5x, this may improve the accuracy at low Platelet count levels.

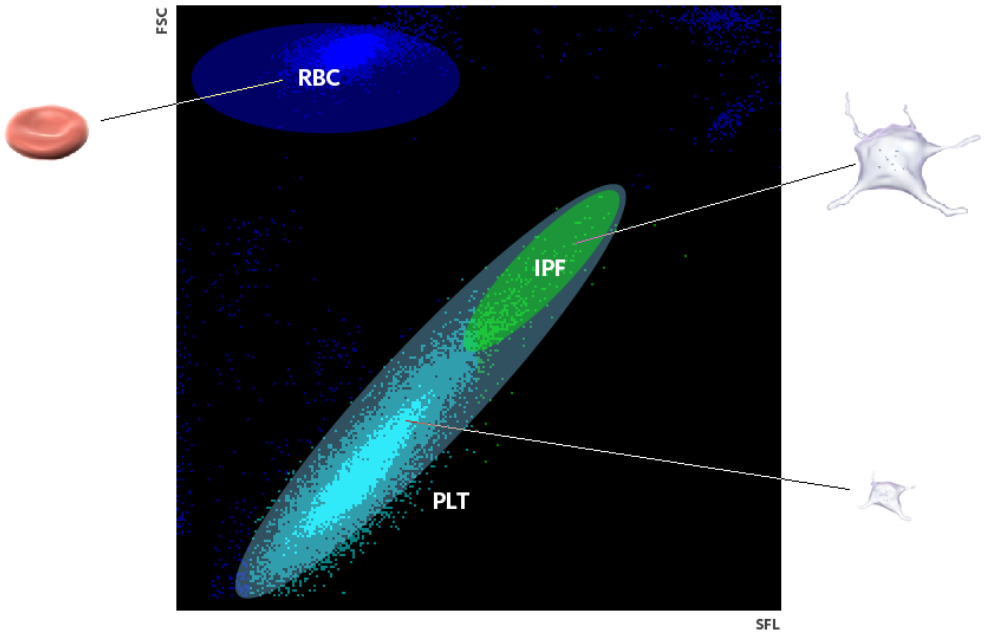


Figure 15- PLT-F Scattergram

Whole Blood Analysis – PLT-F

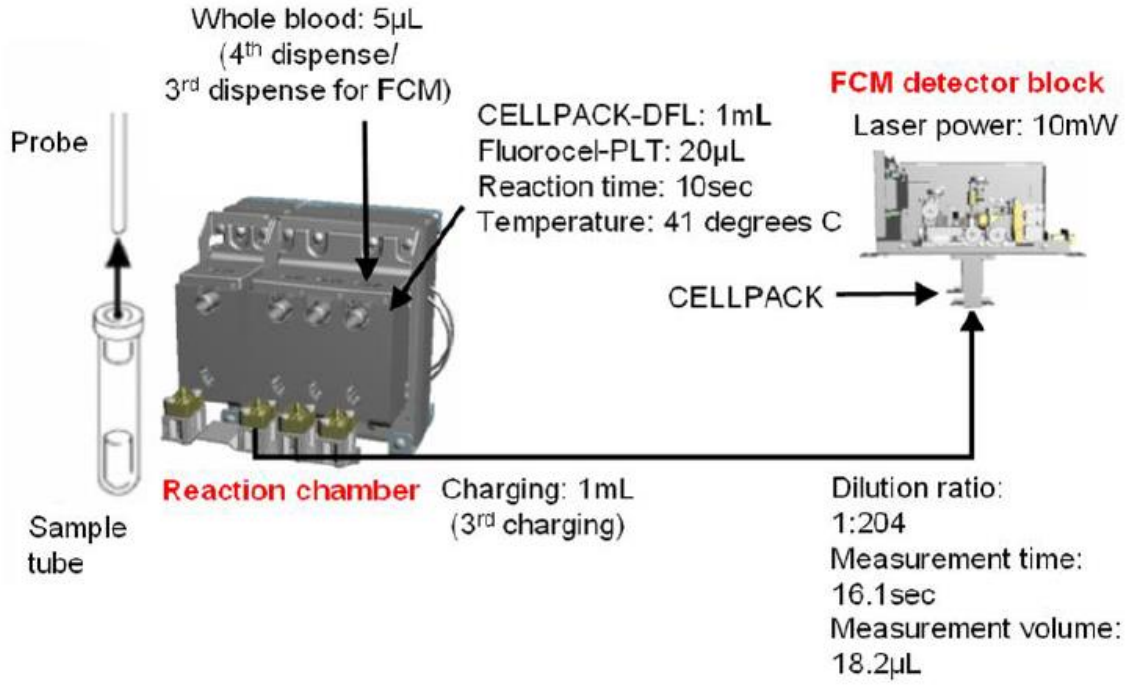


Figure 16- Whole blood Analysis PLT-F

Sheath Flow Direct Current (DC) Detection method

Used in the RBC detector, counts RBC and PLT-I via Hydro Dynamic Focusing (DC detection). At the same time haematocrit (HCT) is calculated via the RBC pulse height detection method.

The RBC and PLT dilution enters the RBC/PLT detector, and is surrounded by sheath reagent (Cellpack DCL). Cells in dilution are hydro dynamically focused to pass through the aperture in single file. A laminar flow at the other side of the detector ensures that cells are not counted twice.

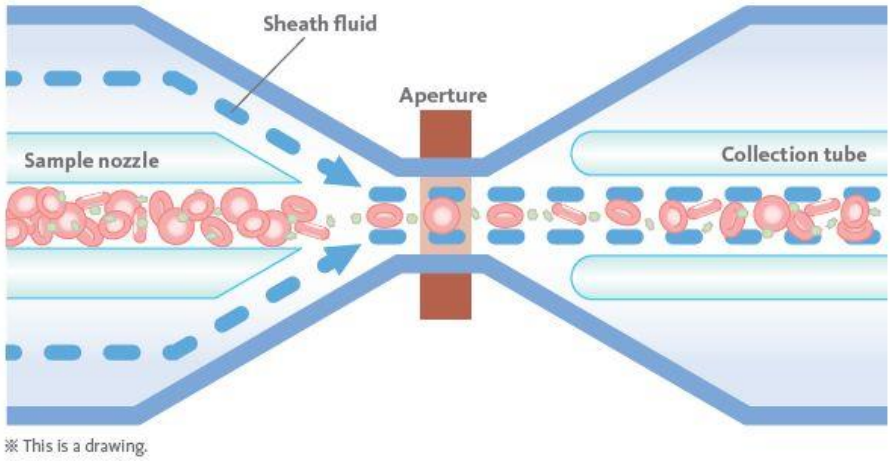


Figure 17- Hydro Dynamic Focused (DC Detection)

As the cells pass through the aperture, they cause an electrical resistance. To compensate, the analyser must increase the voltage to maintain a constant. This increase in voltage adjustment is proportional to the size of the cell, and is recorded as a pulse. The signals are filtered to eliminate interference, and then the RBC and PLT distribution histograms are generated.

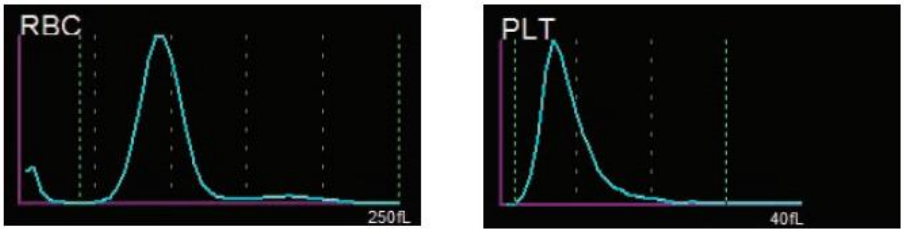


Figure 18- RBC & PLT Distribution Histograms

Whole RBC and PLT Analysis

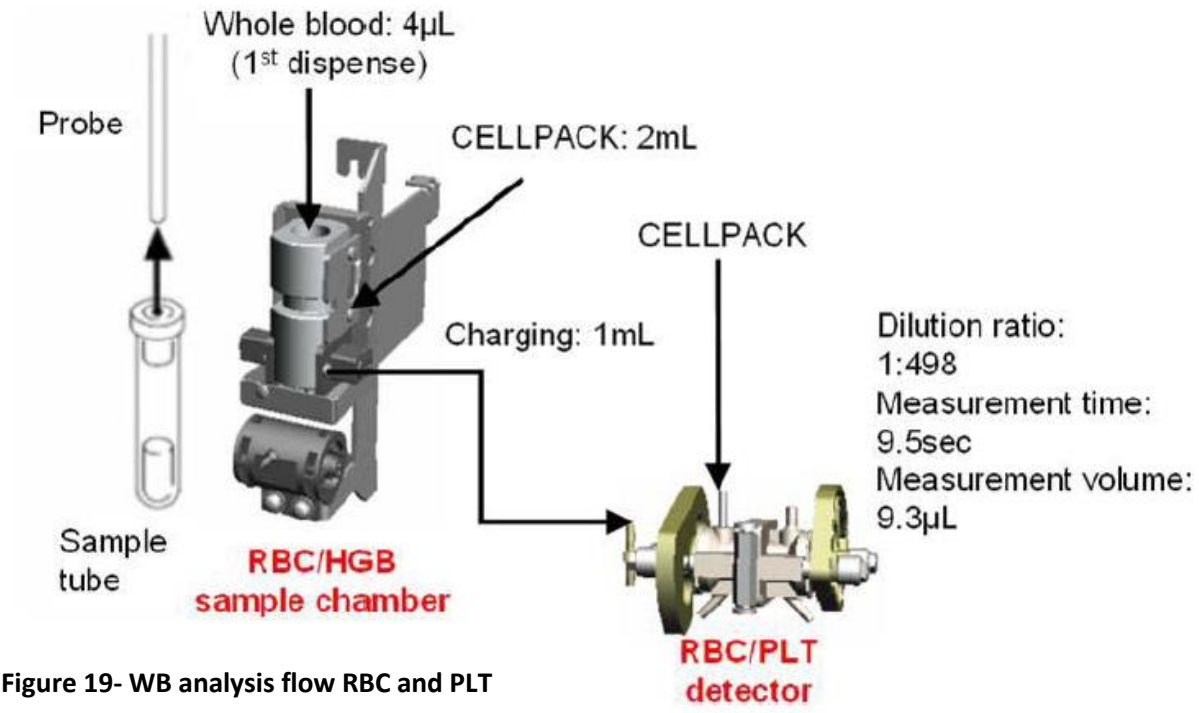


Figure 19- WB analysis flow RBC and PLT

Body Fluid RBC and PLT Analysis

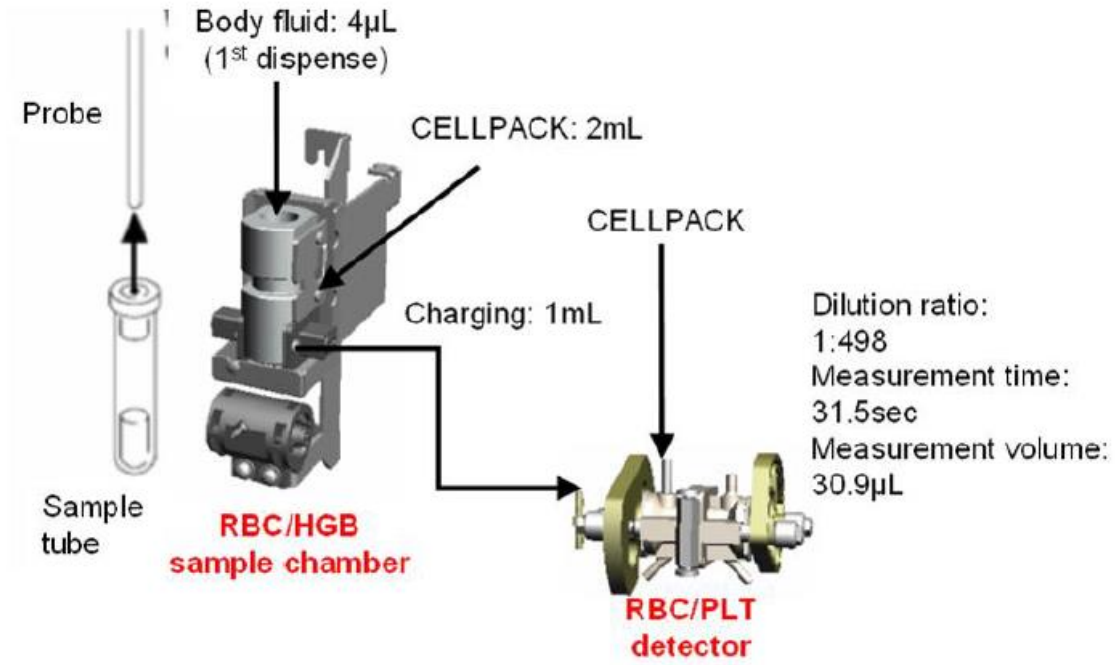


Figure 20- BF analysis flow RBC and PLT

RBC/ HCT

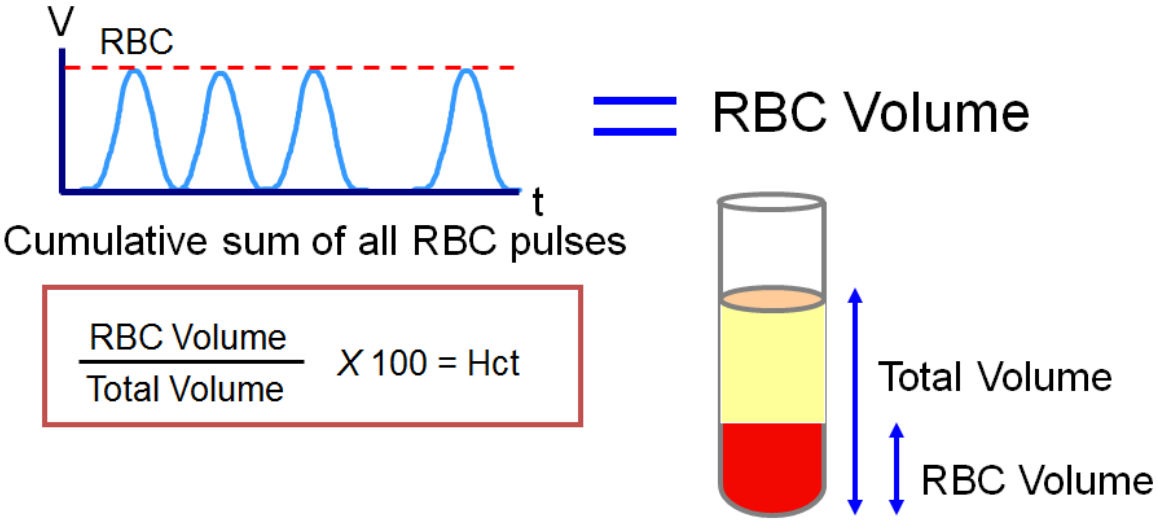


Figure 21- HCT measurement

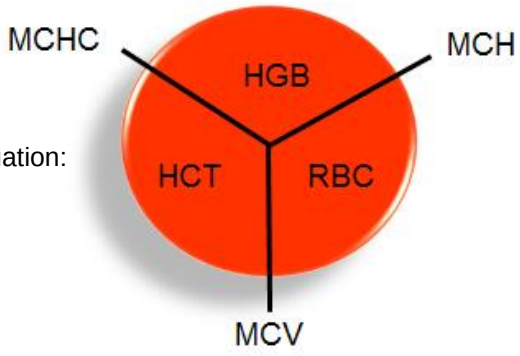
- Cumulative Pulse Height Detection
- Direct measurement, not calculated.
- Resistance change detect as the height of the pulse, proportional to size of RBC.

Calculation of RBC Constants

The red blood cell constants (mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentration) are calculated from the RBC, HGB, and HCT.

Mean Cell Volume (MCV)
The MCV is calculated from the RBC and HCT, using the following equation:

$$MCV\ (fL) = \frac{HCT\ (\%)}{RBC\ (x\ 10^{12}/\ L)}\ x10$$



Mean Cell Haemoglobin (MCH)
The MCH is calculated from the RBC and HGB, using the following equation:

$$MCH\ (pg) = \frac{HGB\ (g/L)}{RBC\ (x\ 10^{12}/\ L)}\ x\ 10$$

Mean Cell Haemoglobin Concentration (MCHC)
The MCHC is calculated from the HCT and HGB, using the following equation:

$$MCHC\ (g/L) = \frac{HGB\ (g/L)}{HCT\ \%}\ x\ 10$$

SLS-Haemoglobin Method

The Sodium-Lauryl-Sulphate (SLS) Haemoglobin method demonstrates good correlation with the ICSH reference method (r=0.99).

The RBC is lysed, and Haemoglobin is liberated. In the reaction chamber the Sodium-Lauryl-Sulphate reagent (Sulfolyser) is added.

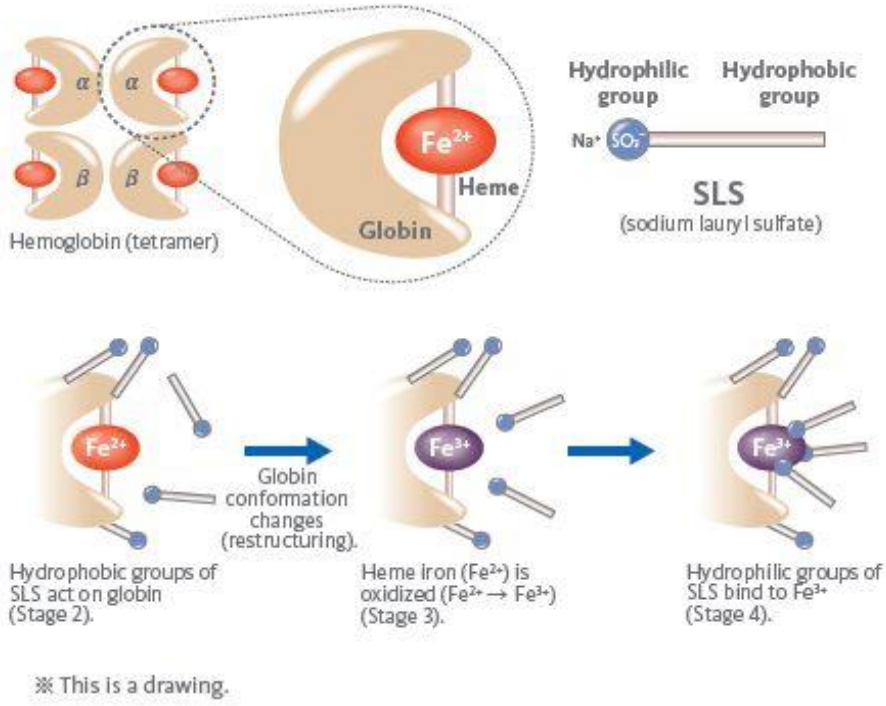


Figure 22- Reaction mechanism of SLS- Haemoglobin Method

The Hydrophobic portion of SLS reacts with globin, and a conformational change occurs exposing the haem unit. Fe^{2+} is oxidised to Fe^{3+}. The hydrophilic group of SLS binds with Fe^{3+} forming a stable reaction product.

The SLS-HGB concentration is measured as light absorbance (555 nM), and is calculated by comparison with the absorbance of the blank diluent sample prior to HGB analysis.

Whole Blood HGB Analysis

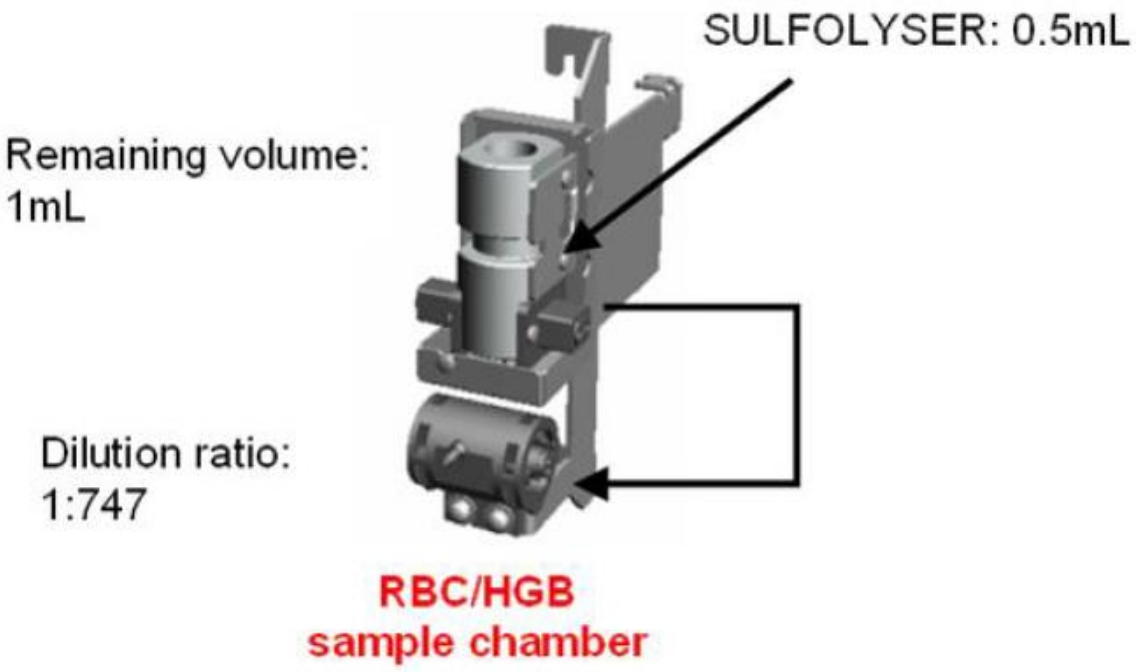


Figure 23- WB analysis flow SLS-HGB

XN Flagging

When analysis data is analysed on the XN IPU; results without an error messages are classified into Positive or Negative based on the pre-set criteria. This classification is displayed at the top left corner of the main view screen in the Data Browser.

NEGATIVE- the sample meets all normal range criteria.

POSITIVE- any one or a combination of the IP messages.

ERROR- an analytical or functional error.

The system bases its judgments on comprehensive surveys of numerical data, particle size distributions, scattergrams, and provides easily-to-understand flags and/or messages indicating the instruments findings. These flags and/ or messages are referred to as "IP (Interpretive Program) messages."

Parameters marking guide:

"*"	Asterisk – Low reliability of data, check by another method such as blood film examination
&	Data has been amended (e.g. WBC and Lymph corrected for NRBC's, PLT-O reported when RBC fragments are present and PLT-F is reported when this channel is utilised during PLT analysis)
@	Data exceeds linearity limits
±	Data exceeding Reference Interval (+ = above, - = below)
----	Analysis cannot be performed
++++	Data exceeds display range
!	Exceeds upper panic limit (upper or lower clinical limits), also value is higher allowable limits for background check

Limitation of Flagging

Low reliability flag in the form of a parameter marking (*) is applied when:

WBC differential have low reliability, results are mark with (*) when:	WB mode, WBC <1.0
	LW mode, WBC <0.4
	PD mode, WBC < 2.0
RBC results have low reliability, results are mark with (*) when:	RBC< 0.50 x 10^{12}/L
PLT-I results has low reliability, results are mark with (*) when:	PLT-I<20 or >1000

IP messages are not performed when:

Judgment for WBC suspect message "Left shift?" Is suppressed when:	Whole blood mode, with WBC results of < 0.50 x 10^{9}/L
DIFF data is masked and no WBC suspect message are available when:	Whole blood mode, with WBC results of < 0.10 x 109/L
DIFF data is masked when:	Pre-dilute mode, with WBC results of < 0.10 x 109/L
Only IP message "RBC Abn Distribution" is performed when:	RBC< 0.50 x 10^{12}/L
Mode of anlaysis Predilute	No Suspect message available
Mode of analysis HPC	Suspect message "Blast/" or "Abn lympho?" not available
Mode of analysis Body fluid	Only WBC flag available is "WBC Abn Scattergram"

IP message judgment is not performed for:

- QC analysis data
- Blank data
- Background check data
- Insufficient blood volume
- Adjustment

Blank data is regarded as;

Blank data is data that meets all of the following conditions:

- **WBC < 0.10 x 10^9/L**
- **RBC < 0.30 x 10^{12}/L**
- **HGB < 10 g/L**
- **PLT < 20 x 10^9/L**

Interpretive (IP) Message

IP messages appear on the sample information tab of the [Sample Explorer] screen, on the main tab of the [Data Browser] screen, and the flag display area of the graph tab.

The IP messages are classified as either **ABNORMAL** or **SUSPECT** flags, and are applied to WBC, RBC, and PLT populations.

For a complete IP message summary, refer to **chapter 11 Instruction For Use manuals (IFU)** relevant to XN systems used section: **"IP message types, meanings, and judgment method"**

Abnormal flagging

- Result out of limit
- Can be define by user

Suspect flagging

- Abnormal morphological finding
- Sample is possibly abnormal

Some flagging conditions are generated from information gathered in more than one channel. For example XN analyser uses combined intelligence for Blast and Abn Lymphocyte flag generation from information in both the WDF and WPC channel.

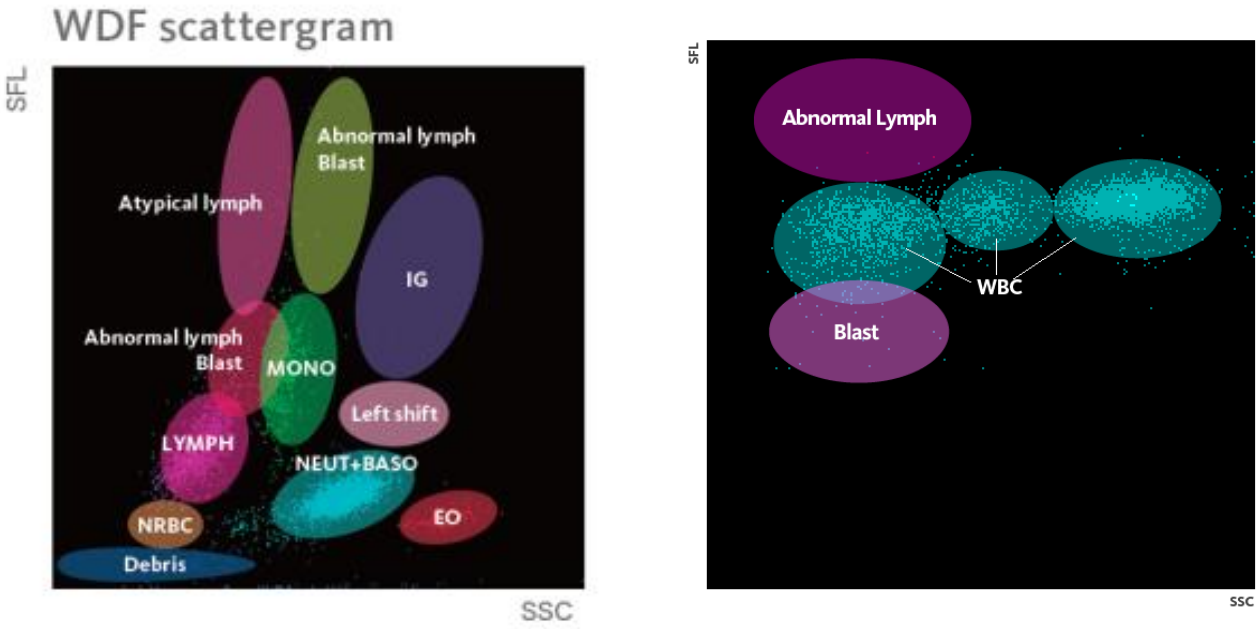


Figure 24- Combined Channel Intelligence Flagging

Q-Flag

Q-Flag tab can be accessed using the Data Browser screen. The [Q-Flag] screen displays the Positive/Negative levels for 10 types of suspect IP messages, as histograms. The displayed information corresponds to the sample operator selected in the analysis data list of the [Sample Explorer] screen.

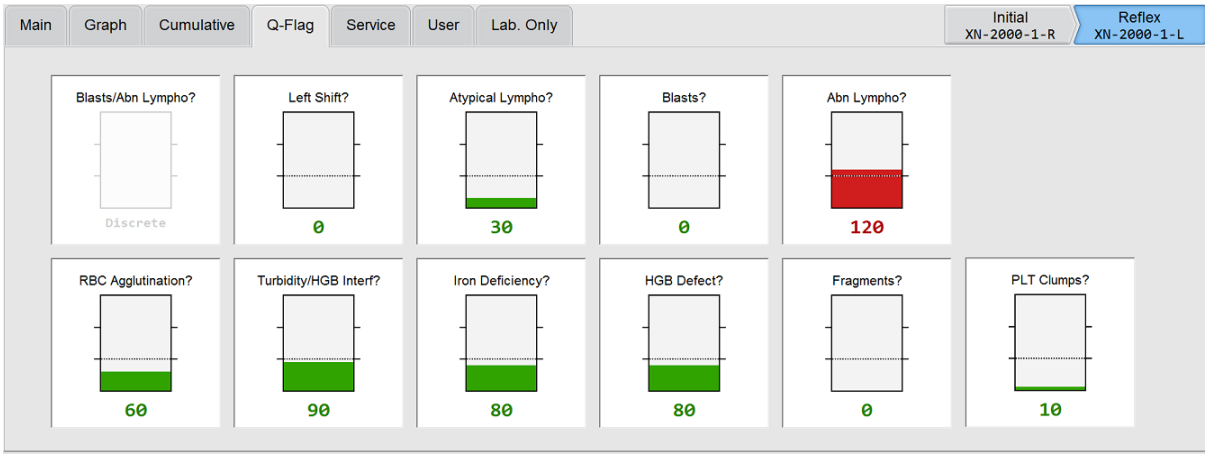


Figure 25- Q-Flag Screen

An arbitrary number is generated based on the undisclosed algorithm to represent activity in the flagging region. This number is compared to a Q-Flag trigger limit. If the number is equal to or larger than the trigger limit the flag is displayed.

Q-Flag displayed values range from 0 to 300, in increments of 10. Values over 100 are determined as Positive. Histogram representation of each result is displayed on the Q-flag tab of the [Sample Browser screen]. In the histogram, Negative results of the sample are displayed in green, and Positive results are displayed in red.

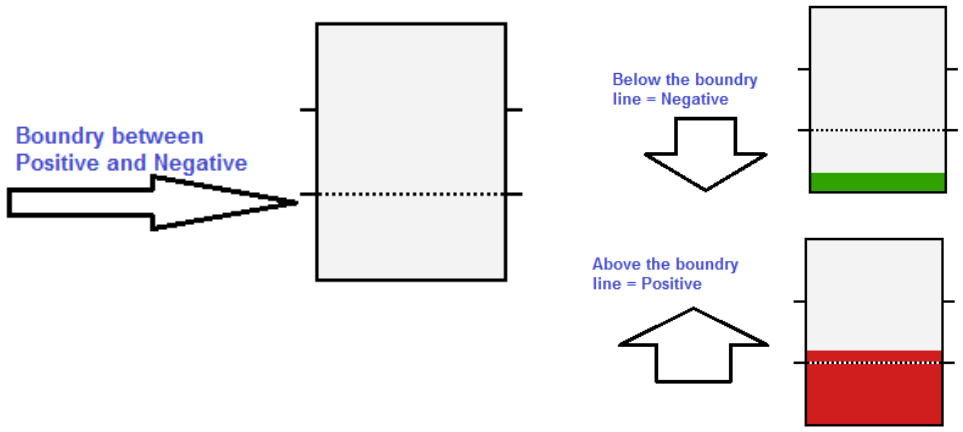


Figure 26- Q-Flag Histogram

In addition, the following may appear in the judgment value position:

[Discrete]: Displayed in grey text. If the parameter used for judgment has not been analysed.

[Error]: If judgment impossible.

Blank: If prerequisite for judgment was not met. Also, if the suspect judgment was not performed due to blank data, etc.

Depending on the instrument type following 10 IP message and Q-Flag are generated:

Q-Flag	Message	Meaning
WBC	***Blasts/ Abn Lympho***	*Possibility of blasts or abnormal lymphocytes*
	Blasts?	*Possibility that blasts are present*
	Left Shift?	*Possibility of left shift*
	Abn Lympho?	*Possibility abnormal lymphocytes*
	Atypical Lympho?	*Possibility atypical lymphocytes*
RBC	***RBC Agglutination?***	*Possibility RBC agglutination*
	Turbidity /HGB Interf?	*Possibility HGB interference by chylemia*
	Iron Deficiency?	*Possibility of Iron deficiency anaemia*
	HGB Defect?	*Possibility of HGB abnormality*
	Fragments?	*Possibility of fragmented RBCs*
PLT	***PLT Clumps?***	*Possibility of PLT clumps*

Table 2- Q-Flag and relevant IP Message

Information:

Labs that experience large numbers of false positive flags can perform a study to adjust the Q-flag trigger limits. It is important that the Q-flag review procedure be carried out in full cooperation with Roche Diagnostic Australia staff. For more information, please see your Application Specialist trainer or call the Technical Hotline.

Section 3: Body Fluid, HPC and hSA

Body Fluid analysis

Body fluid (BF) is optional and can be activated on all XN instruments.

On instruments which offer this option, body fluid analysis for cerebrospinal fluid, serous (peritoneal and pleural) and Synovial fluids can be performed in 90sec.

Following selection of the Body Fluid mode, XN system will automatically run an extended background to reduce carryover before it is ready for body fluid analysis. Body fluid results obtained includes; total WBC, RBC counts as well as an individual count for polymorphonuclear (PMN) and mononuclear (MN) cell populations.

WBC results are generated using WDF channel, RBC count using RBC/PLT channel.

Automatic Background check preparation perform by the XN must result in background check under the allowable limits as stated:

WBC-BF	*0.001 X 10^9/ L or less*
RBC-BF	*0.003 x 10^{12}/ L or less*

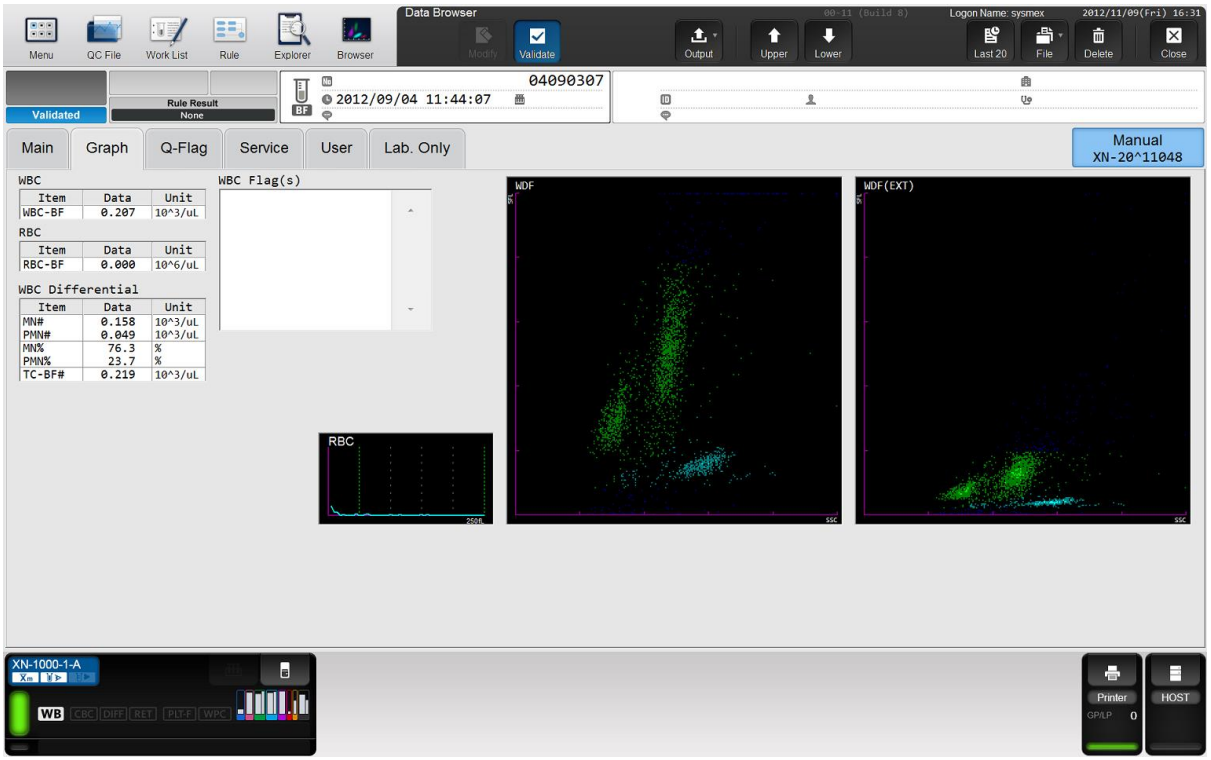


Figure 27- Body Fluid Screen

HPC analysis (only applicable for XN-20 module with activated HPC license)

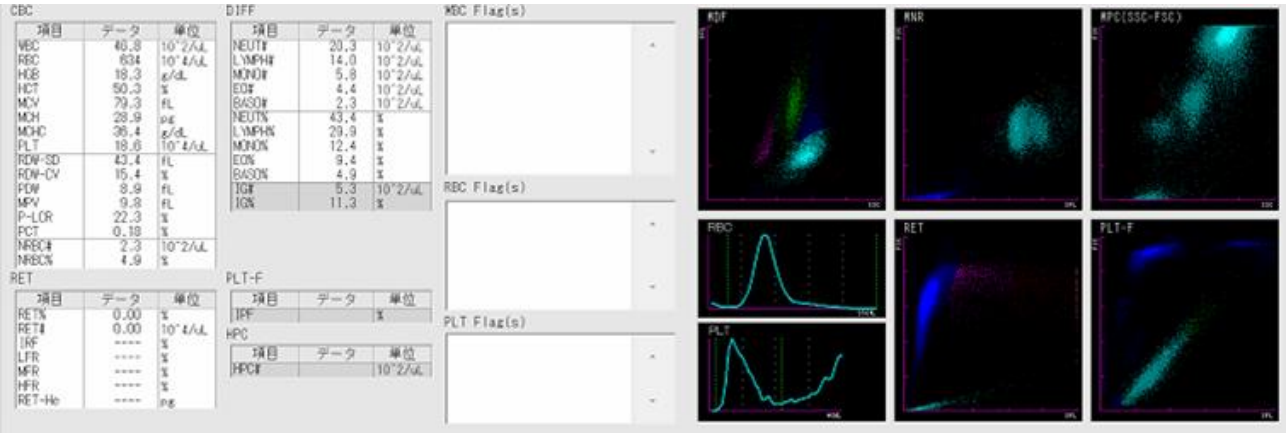
Haemopoetic Progenitor Cells (HPC) is an optional mode of analysis and can be activated on all XN-20 instruments.

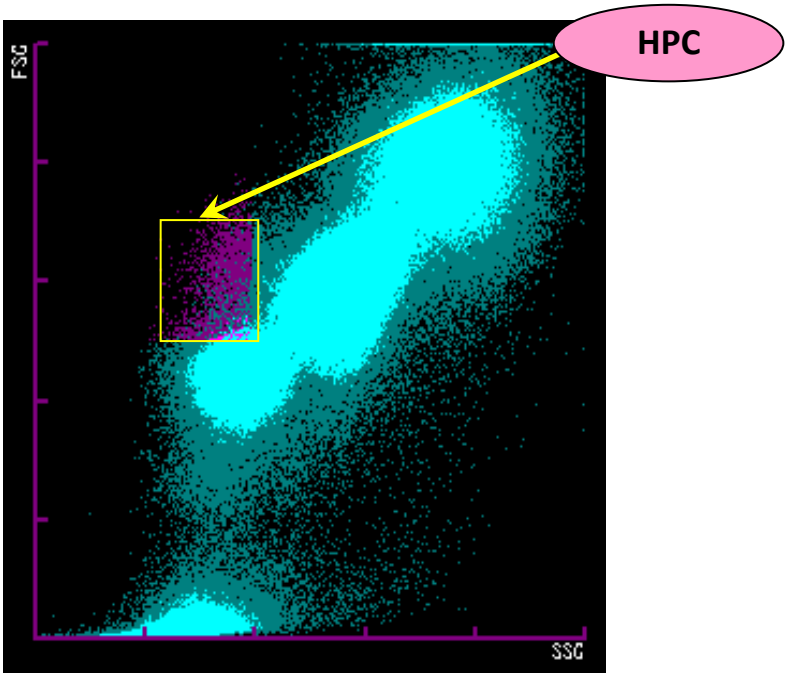
On instruments which offer this option, HPC# is measured by the WPC channel.

HPC is only available in the manual analysis, and can be performed in less than 4 minutes.

Instrument will aspirate the sample 2 times, in total the aspiration volume will be 190µL:

- 1st aspiration: 88µL = Whole blood mode measurement with all discrete selected
- 2nd aspiration: 102 µL = 4x WPC channel measurement





hSA analysis (only applicable for XN module with activated BF and RET licenses)

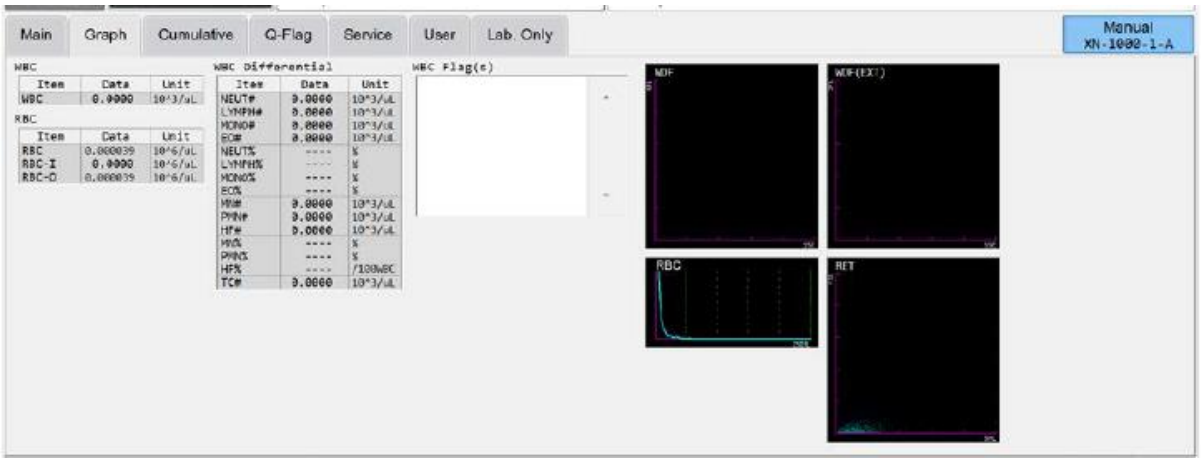
High sensitivity analysis (hSA) is optional and can be activated on all XN instruments.

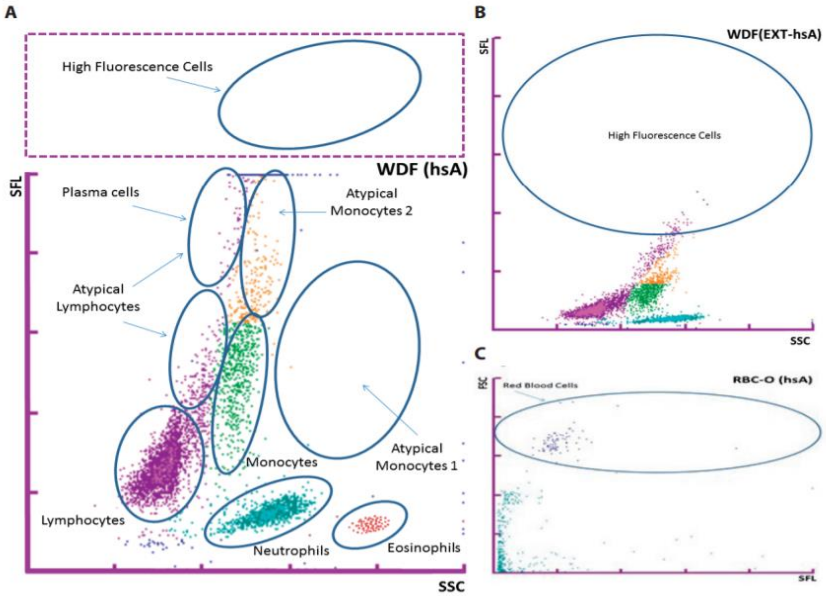
The hsA mode is a measurement function that allows higher precision calculation by increasing the measurement volume, compared to WB mode, WBC: 20 times more volume; RBC (RBC-O): 170 times more volume. WBC results are generated using WDF channel, RBC-I count using RBC/PLT channel and RBC-O count using RET channel.

On instruments which offer this option, hSA analysis for cerebrospinal fluid, blood derivatives, concentrated RBC derivatives and plasma derivatives can be performed in 90sec.

Automatic Background check preparation perform by the XN must result in background check under the allowable limits as stated:

WBC	*0.00100 X 10^3/ µL or less*
RBC-I	*0.0030 x 10^6/ µL or less*
RBC-O	*0.000100 x 10^6/ µL or less*





Section 4: Practical Assessments

Knowledge Review

☐ XN analyser applies 3 primary analysis principles these are:

1)…….

2)…….

3)…….

☐ In Flow Cytometry method of detection laser emits light at what absorbance:

a) 663nm

b) 633nm

c) 555nm

☐ The diluted samples go through the centre of laser flow cell assisted by which principle:

a) Column Flow Dynamics

b) Fluidic Hydro Laminar Flow

c) Hydrodynamic Focussing

☐ SLS method of HGB measurement applies which principle of detection:

a) Sheath Flow DC

b) light absorbance at 555 nM

c) Radio Frequency and light absorbance at 585 nm

☐ Side Fluorescent Light signals indicates:

a) Cell size

b) Cellular internal structure

c) Types and amount of Nucleic Acids and organelles information

☐ Classification of cell population in WDF Channel is based up on two algorithms and they are:

A……C ……A……. learning algorithm &

S……..A ……..FL ……A ……..S ……..

☐ Sysmex Adaptive Flagging Algorithm based on Shape recognition, is used in classification of which cell populations:

a) RBC and PLT

b) Abnormal Lymphocyte and Monocyte

c) Retic and PLT-O

☐ WPC channel is a channel for detecting which cell types (only applicable to XN-20 customers)

1) ……

2) ……..

☐ What is the temperature of WPC reaction chamber (only applicable to XN-20 customers)

a) 44 degree C

b) 25 degree C

c) 34 degree C

☐ List the Clinical Parameters obtained from Retic channel:

……

☐ The sum of MFR and HFR = ……..

☐ Which detection principle is applied in the PLT-F channel:

……

☐ Which Fluorescent dye is used in PLT-F fluorocell reagent:

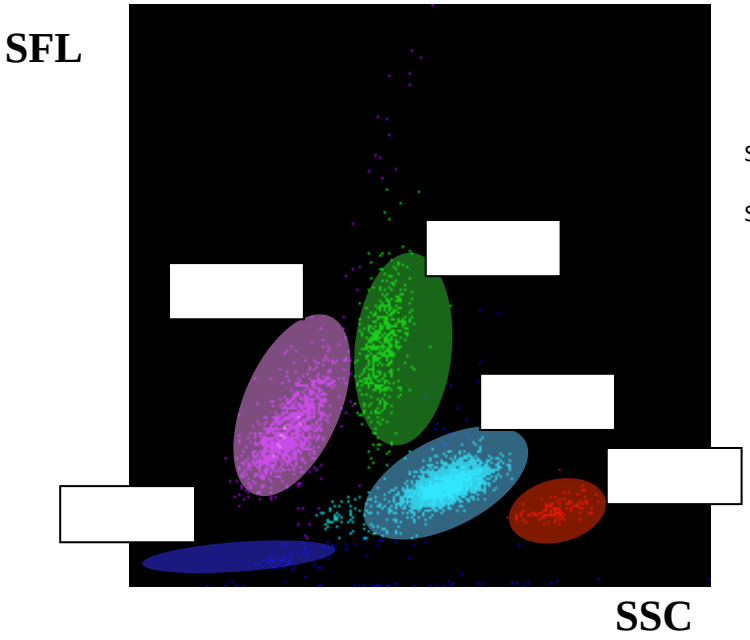
a) Polymethine Dye

b) Oxazine Dye

c) Bromothymol blue dye

☐ Label Cell clusters and describe the type of light used on X and Y axis of the scattergrams (what cellular information is used to determine the cell type):

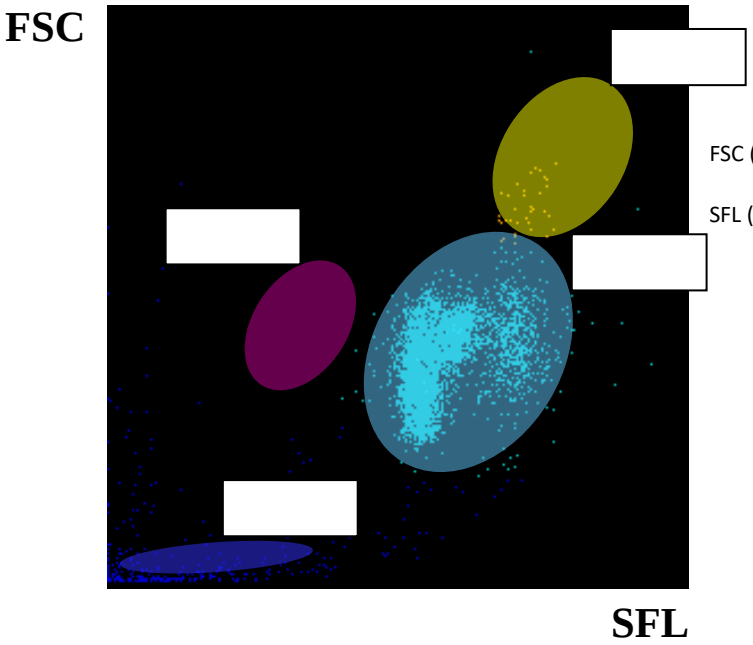
WDF Scattergram



SFL (Side Fluorescent Light)=.....

SSC (Side Scattered Light)=.....

WNR Scattergram



FSC (Forward Scattered Light)=.....

SFL (Side Fluorescent Light)=.....

☐ Describe what following Parameters markings mean:

"*" ……

& ……

---- ……

@ ……

! ……

Trainee Signature…… Date ……

Trainers Signature…… Date ……