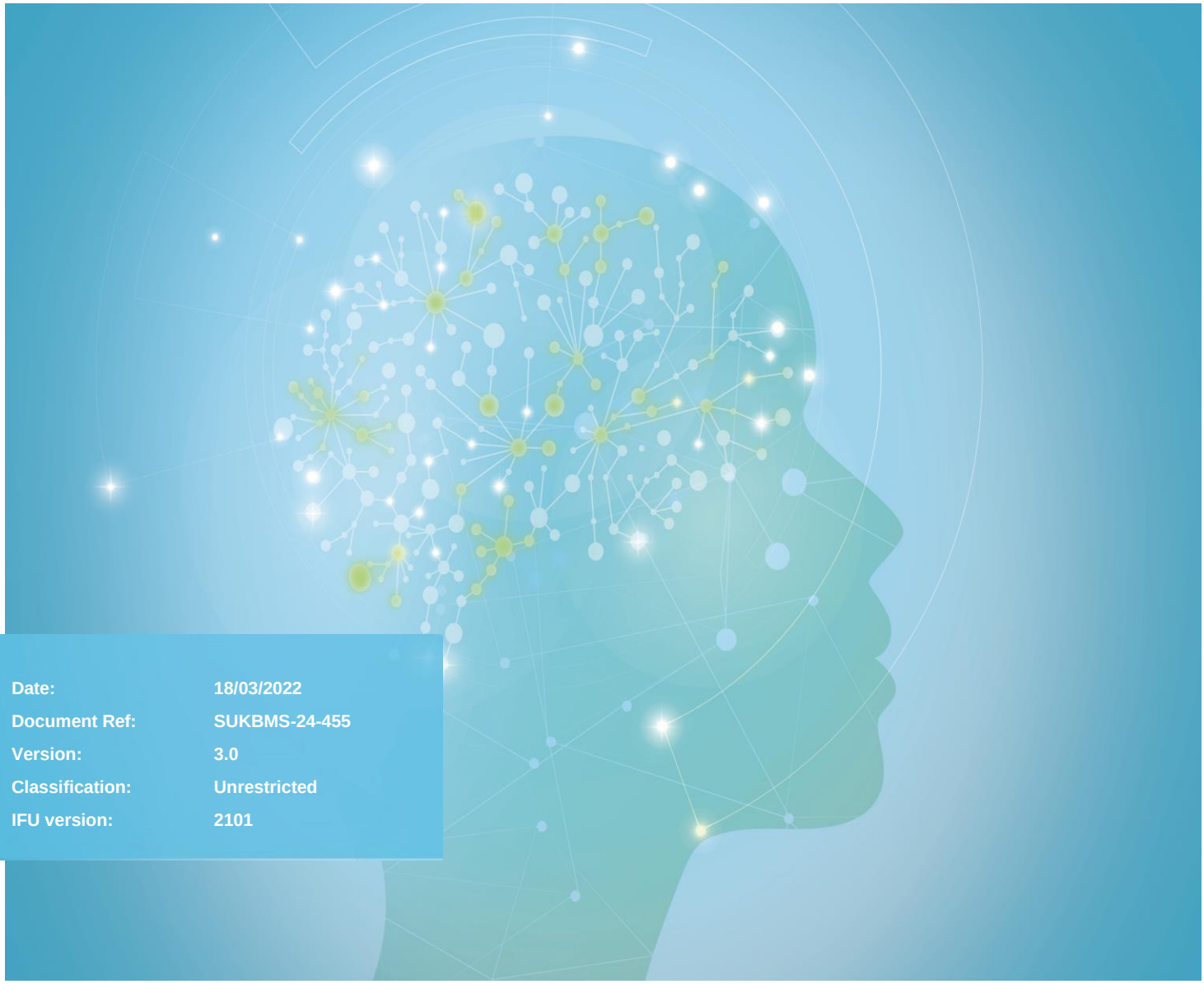
Lighting the way with diagnostics

Analysis Principles Training Workbook

XN-Series



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Disclaimer

Please note, the information contained in training resources provided by Sysmex should not be used as an alternative to your sites Standard Operating Procedure (SOP)/Contract. If you have any particular questions regarding any site specific use of reagents, consumables and/or equipment please contact your Management Team.

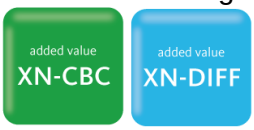
Revision History

Revised section	Alteration	Name	Date
All	New document to replace the following documents: - XN-9000 Onsite Training Workbook - XN-9100 Onsite Training Workbook - XN-Series Training Workbook	N.Bowen	June 2020
RET Channel	Erythropoiesis image updated	N.Bowen	25th June 2020
WPC Channel	WPC Scattergram images updated	N.Bowen	25th June 2020
Reagents	Reagent table updated to reflect XN-Series Routine Use Training Workbook	N.Bowen	July 2020
Title	Name changed from XN-Series Theory Training workbook to: - XN-Series Analysis Principles Training Workbook	N Bowen	March 2022
WDF Channel	Information included on how neutrophil count is calculated	N Bowen	March 2022

Reference Documents

Document title	Version	Date
XN-1000 IFU	2101	January 2021
XN-2000 IFU	2101	January 2021
XN-3000 XN-3100 IFU	2101	January 2021
XN-9000 XN-9100 IFU	2003	March 2020

Overview of XN-Series

Facts and figures	
Analysers	XN-10 and XN-20
Configurations	XN-1000, XN-2000, XN-3x00 and XN-9x00
Analytical components	Standard: 28 diagnostic parameters as standard  Optional: 16 optional diagnostic parameters 
Technologies	Spectrophotometry, Sheath flow (DC) detection and Fluorescence flow cytometry
Modes of analysis	Whole blood (WB) mode Low WBC (LW) mode Pre-dilution (PD) mode Body fluid (BF) mode HPC mode
Aspiration methods	System Mode (XN-9x00 configurations only) Sampler analysis Manual analysis (Closed/open/micro/raised bottom tube)
Throughput	100 samples per hour (CBC+DIFF)
Quality control	XN CHECK XN CHECK BF
Aspiration volumes	88µl (WB, LW, BF modes) 70 µl (PD mode)
Analysis range (whole blood mode)	WBC 0.00 to 440 $x10^3$/µL RBC 0.00 to 8.60 $x10^6$/µL Hb 0.0 to 260g/L HCT 0.0 to 75.0% PLT 0 to 5000 $x10^3$/µL NRBC# 0.00 to 20.00 $x10^3$/µl NRBC% 0.0 to 600.0/100WBC RET% 0.00 to 30.00% RET# 0.0000 to 0.7200 $x10^6$µL

Reagents On-board the XN-Series

There are a total of 12 different reagents that can be used on XN-Series analysers (varies due to configuration), each having a different purpose. A summary can be found below describing the reagents name, cycle per container and onboard stability.

Reagent Name	Cycles Per Container (approx.)	On board Stability (Days)	Parameters Produced
CellPack DCL (10L)	200	60	Red cell and platelet parameters*
CellPack DST (10L)	5,000	60	
Sulfolyser (5L)	10,000	90	Haemoglobin
Lysercell WNR (5L)	2,000	60	Total white cell count, basophil count and nucleated red cell count
Fluorocell WNR (82ml)	4,000	90	
Lysercell WDF (5L)	3,333	90	Neutrophils, lymphocytes, monocytes, eosinophils and Immature granulocytes
Fluorocell WDF (42ml)	2,000	60	
Lysercell WPC (1.5L)	1,000	90	Blast? and Abnormal Lympho? flags
Fluorocell WPC (12ml)	500	90	
CellPack DFL (1.5L)	1,500	60	Used in conjunction with Fluorocell RET and Fluorocell PLT
Fluorocell RET	500	90	Reticulocytes and optical platelet count
Fluorocell PLT	500	90	Fluorescent platelet count and immature platelet fraction

* CellPack DCL/CellPack DST are also used in all channels for hydrodynamic focussing of cells and for rinsing the lines between samples.

Analysis Principles and Parameter Production

The XN-Series analysers utilise 3 primary analysis principles:

1. Sheath Flow (DC) Detection – RBC and PLT analysis.
2. Spectrophotometry – SLS Haemoglobin Method (cyanide-free HGB analysis).
3. Fluorescence Flow Cytometry (FCM) – a semiconductor laser (633nm).

Sheath Flow Direct Current (DC) Detection

Sheath flow (DC) detection is only used in the XN-CBC channel on the XN-Series analysers. The XN-CBC channel is a standard channel on both XN-10 and XN-20 analysers.

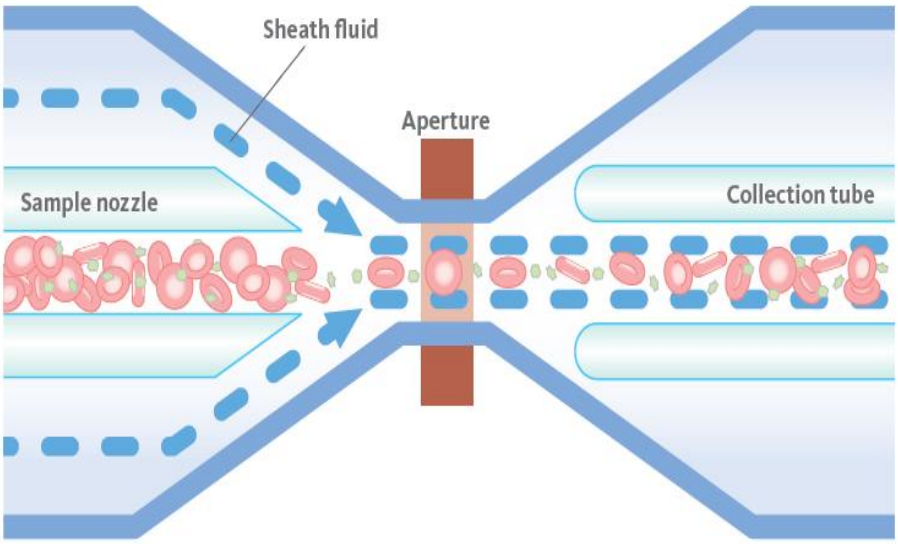
CellPack DCL/CellPack DST

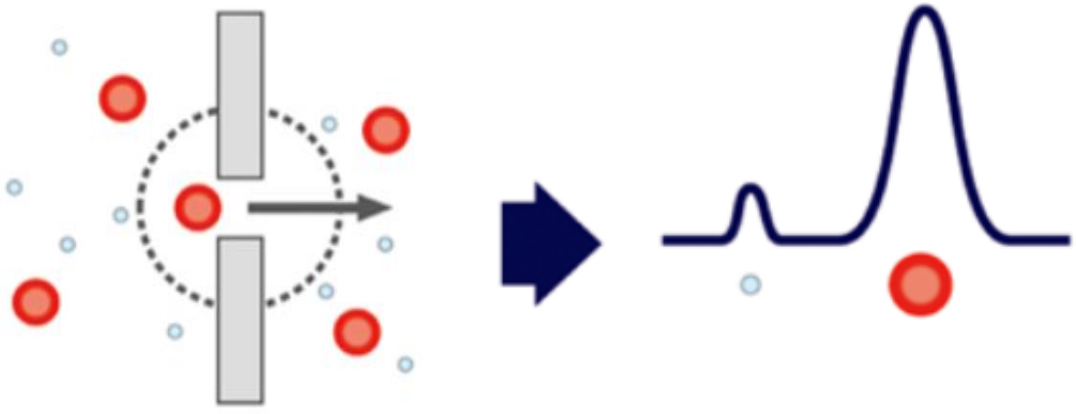
CellPack DCL is a ready to use reagent for use with Sysmex analysers. CellPack DST is a concentrated form of CellPack DCL for use with a reagent preparation unit, where it is diluted with deionised water to produce the same concentration as Cellpack DCL, which is then supplied to the analyser. One 10 litre box of CellPack DST is equivalent to 25 10 litre boxes of CellPack DCL.

CellPack DCL/CellPack DST is used in the XN-CBC channel to produce RBC and platelet parameters via Sheath Flow (DC) Detection

CellPack DCL/CellPack DST is used as a sheath fluid for hydrodynamic focussing in sheath flow (DC) detection and in the flow cytometry detector. It also performs several other functions on the XN-Series analysers; it protects RBCs and platelets, prevents backflow, acts as an isotonic diluent and rinses the lines of the analyser between each sample.

The XN-Series analysers use sheath flow (DC) detection for producing RBC and platelet parameters using CellPack DCL/CellPack DST. The RBC and PLT dilution is injected into the RBC/PLT detector. The sample dilution passes through the middle of the aperture, assisted by the hydrodynamic focussing principle where laminar flow ensures that cells are not counted twice. As cells pass through the aperture, they cause an electrical resistance, which is recorded as an impedance pulse. The size of the cell is proportional to the pulse height (as shown in the diagram below).





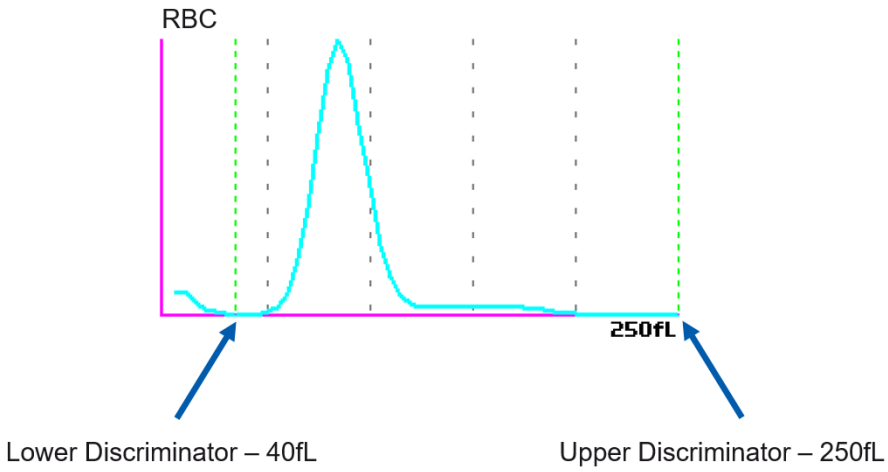
Aperture

Blood Cell Pulse

The RBC and PLT histograms are generated from this detection principle. The x-axis on the histogram relates to the size of the cell and the y-axis relates to the number of cells counted.

RBC Histogram

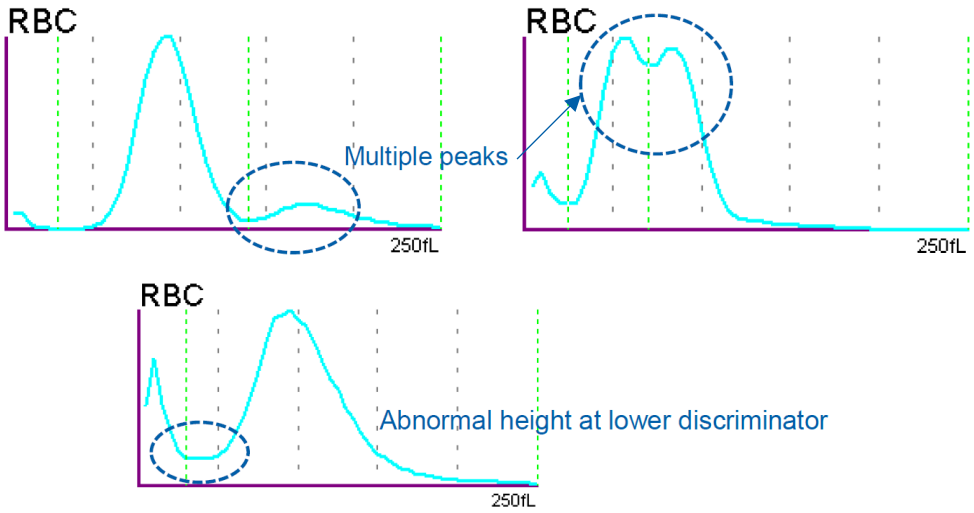
Normal RBC Histogram



The upper discriminator and lower discriminator values are fixed, and the analyser uses these to determine what cells to count as a RBC. Each black dotted marker decreases by 50fL, starting at the upper discriminator, and can be used to help determine the size of the RBC population.

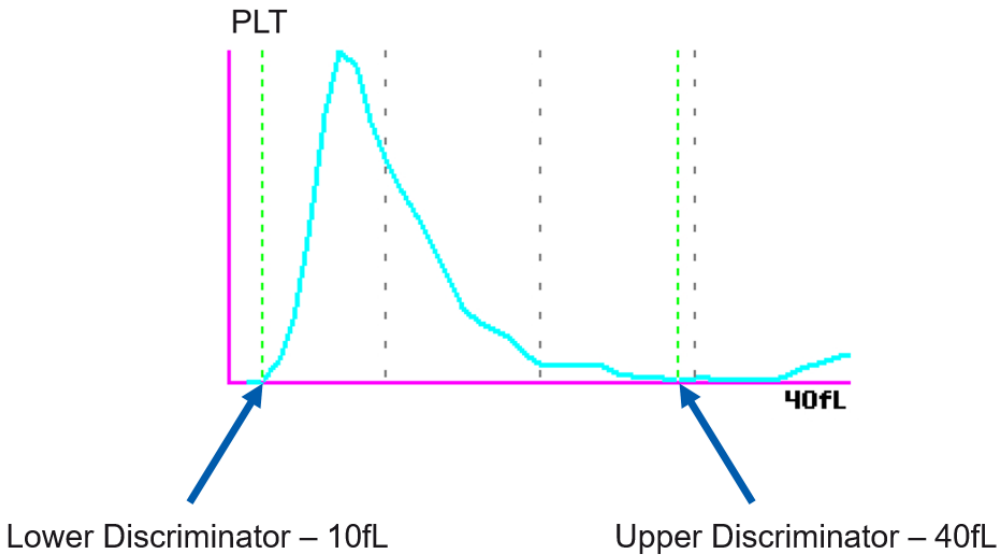
Abnormal RBC Histogram

Below shows examples of abnormal RBC histograms that may be generated by the XN-Series analysers. Any abnormal RBC histogram pattern that is generated will result in the RBC Abn Distribution flag being generated.



PLT Histogram

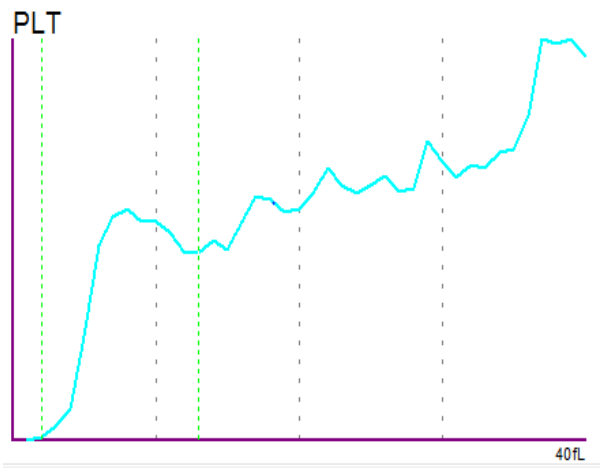
Normal PLT Histogram

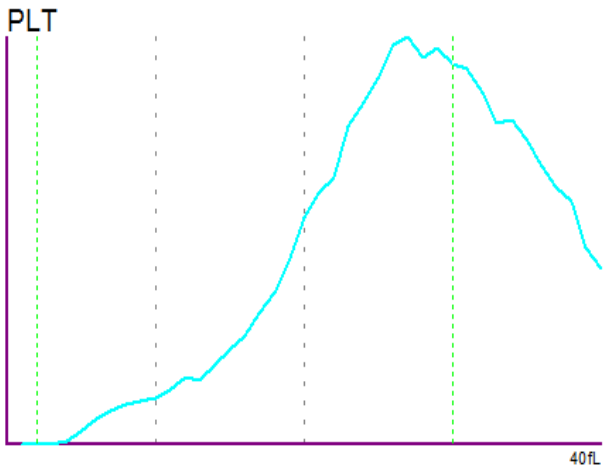


The upper discriminator and lower discriminator values are fixed, and the analyser uses these to determine what cells to count as a PLT. Each black dotted marker decreases by 10fL, starting just after the upper discriminator, and can be used to help determine the size of the PLT population.

Abnormal PLT Histogram

Below shows examples of abnormal PLT histograms that may be generated by the XN-Series analysers. Any abnormal PLT histogram pattern that is generated will result in the PLT Abn Distribution flag being generated.





added value
XN-CBC

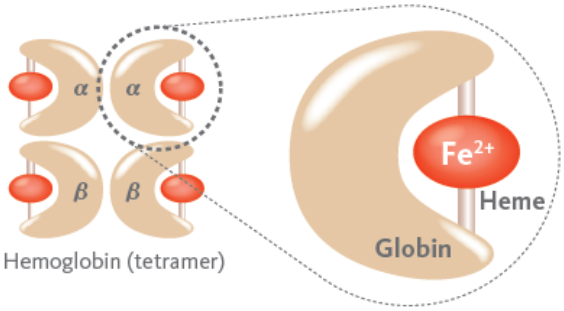
Spectrophotometry

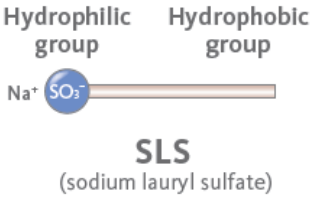
Spectrophotometry is only used in the XN-CBC channel on the XN-Series analysers.

Sulfolyser

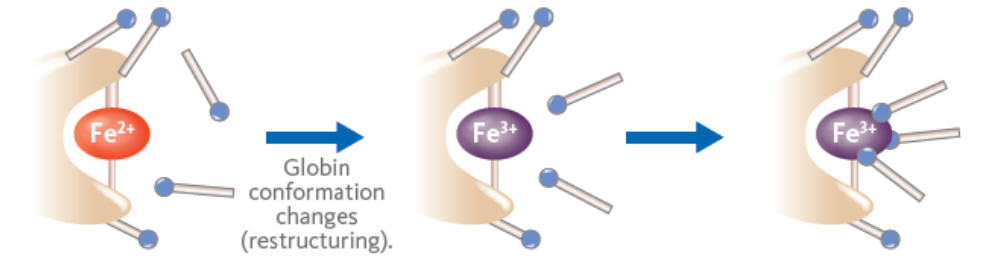
Sulfolyser (SLS) is used in the XN-CBC channel to produce the haemoglobin result via spectrophotometry.

They key component of Sulfolyser is ‘Sodium Lauryl Sulfate’. This molecule has hydrophobic / hydrophilic portions (see diagram), which is significant for its purpose.

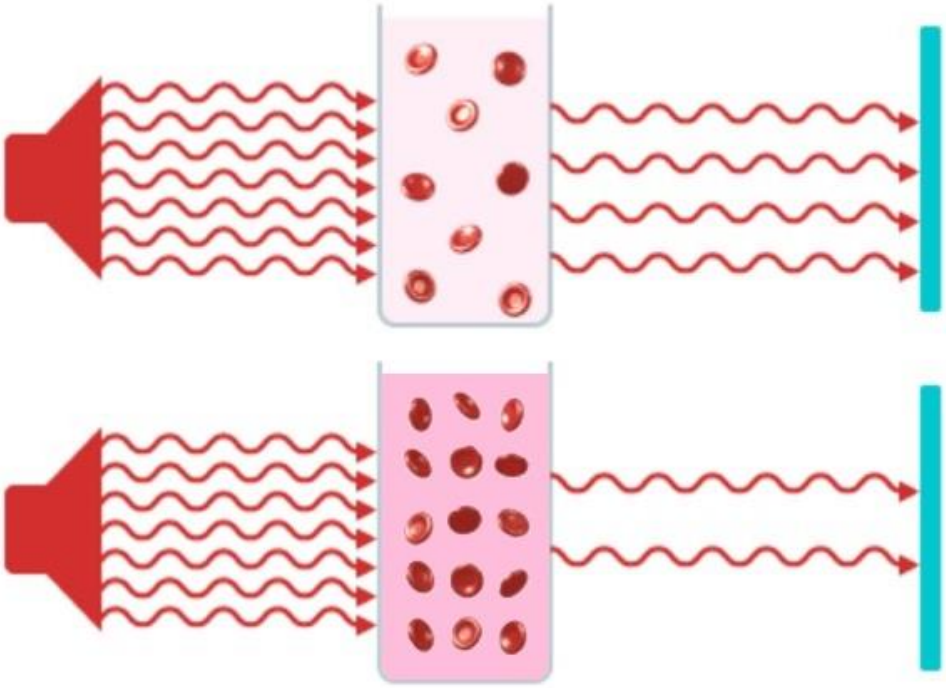




When mixed with the sample SLS causes red cell lysis, which allows the hydrophobic portion to attach to the globin molecule. This causes a conformational change in the globin molecule which leads to the haem group being oxidised from Fe^{2+} to Fe^{3+}. Following oxidation, the hydrophilic portion of SLS attaches to the haem group.forming a stable, coloured complex.



The newly formed stable coloured complex is then passed through a spectrophotometer and read at 555nm. A blank is taken using CellPack DCL/DST, the difference between the blank and the reaction complex is directly proportial to the haemoglobin concentration.



Erythrocyte Indices

Erythrocyte Indices: mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) are calculated from the RBC, HGB (g/dL) and HCT parameters generated in the XN-CBC channel:

MCV (fL) = [HCT %) ÷ RBC ($x10^6$/µl)] x 10

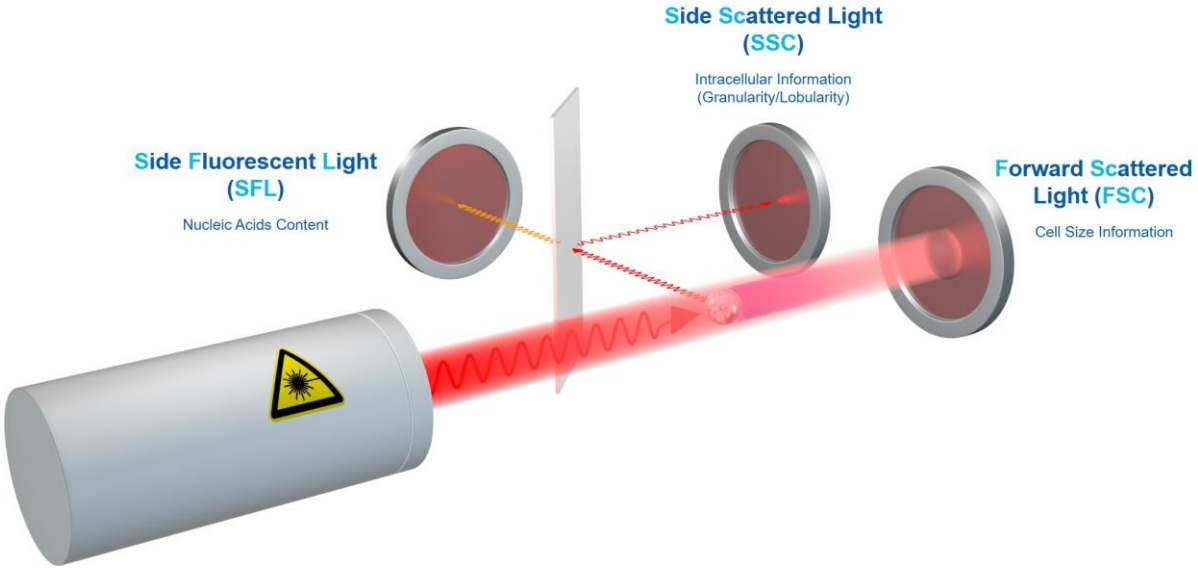
MCH (pg) = [HGB (g/dL) ÷ RBC ($x10^6$/µl)] x 10

MCHC (g/dL) = [HGB (g/dL) ÷ HCT (%)] x 100

added value XN-CBC | added value XN-DIFF | added value RET | added value PLT-F | added value WPC

Fluoresence Flow Cytometry

The XN-Series analysers use a semi-conductive laser that emits light in the red region of the spectrum at 633nm. The laser interrogates each cell individually and then detectors collect the light from the interrogation as shown in the diagram below.



Sample dilutions are hydrodynamically focussed through the centre of the flow cell using CellPack DCL, in the same way as the cells are hydrodynamically focussed during sheath flow (DC) detection. As cells pass through the laser beam, forward scattered light (FSC) is generated. A fluorescent polymethine dye is added in the reaction channel and binds to DNA/RNA and organelle content within cells. A dichroic mirror is used to separate the fluorescent light emitted, known as side fluorescent light (SFL) from the side-scattered light (SSC) which is emitted at a different wavelength.

The light detected provides 3 different types of information on the cell:

1. Forward Scattered Light (FSC) = Cell Size (volume)
2. Side Scattered Light (SSC) = Granularity and Lobularity (Intracellular complexity)
3. Side Fluorescent Light (SFL) = RNA/DNA and cell organelle content

Based on the three different types of light signal detected each cell produces a unique “cell signature” or position on the scattergram, as it passes through the laser beam. 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.

All XN-Series channels make use of this technology:

- XN-CBC (WNR) Channel
- XN-DIFF (WDF) Channel
- XN-RET Channel
- XN-PLT-F Channel
- XN-BF (Body Fluid) Mode
- XN-WPC Channel

added value
XN-CBC

XN-CBC (WNR) Channel

The XN-CBC (WNR) is a standard channel on both XN-10 and XN-20 analysers. Fluorescence flow cytometry within the XN-CBC (WNR) Channel is used for enumeration of WBCs (non-basophil), basophils and NRBCs, using CellPack DCL/DST (as an isotonic diluent), Lysercell WNR & Fluorocell WNR.

Reagents

Lysercell WNR

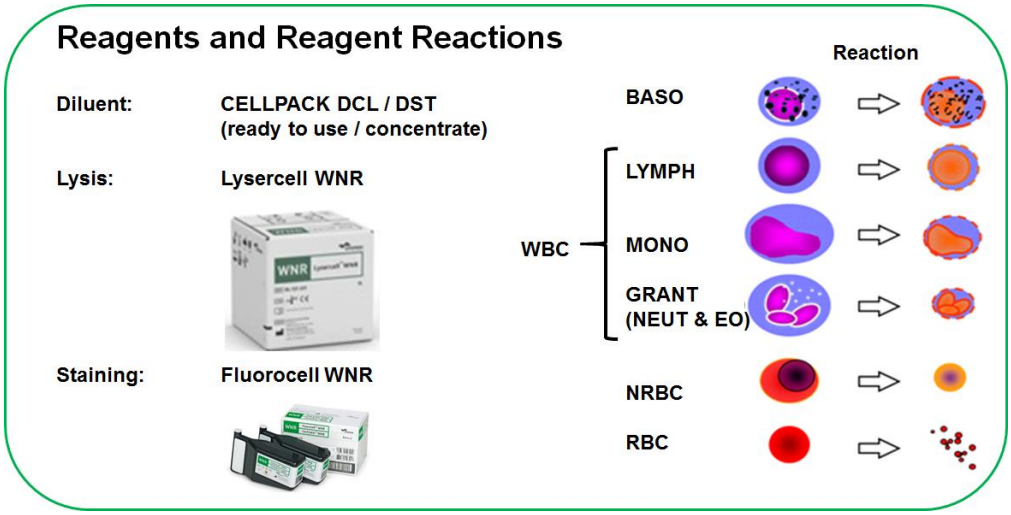
The Lysercell WNR reagent haemolyses red blood cells and leaves NRBCs as bare nuclei.

It shrinks all of the WBCs except the basophils which remain in a near native state.

At the same time the membranes of WBCs are made permeable to allow the Fluorocell WNR to enter the cells.

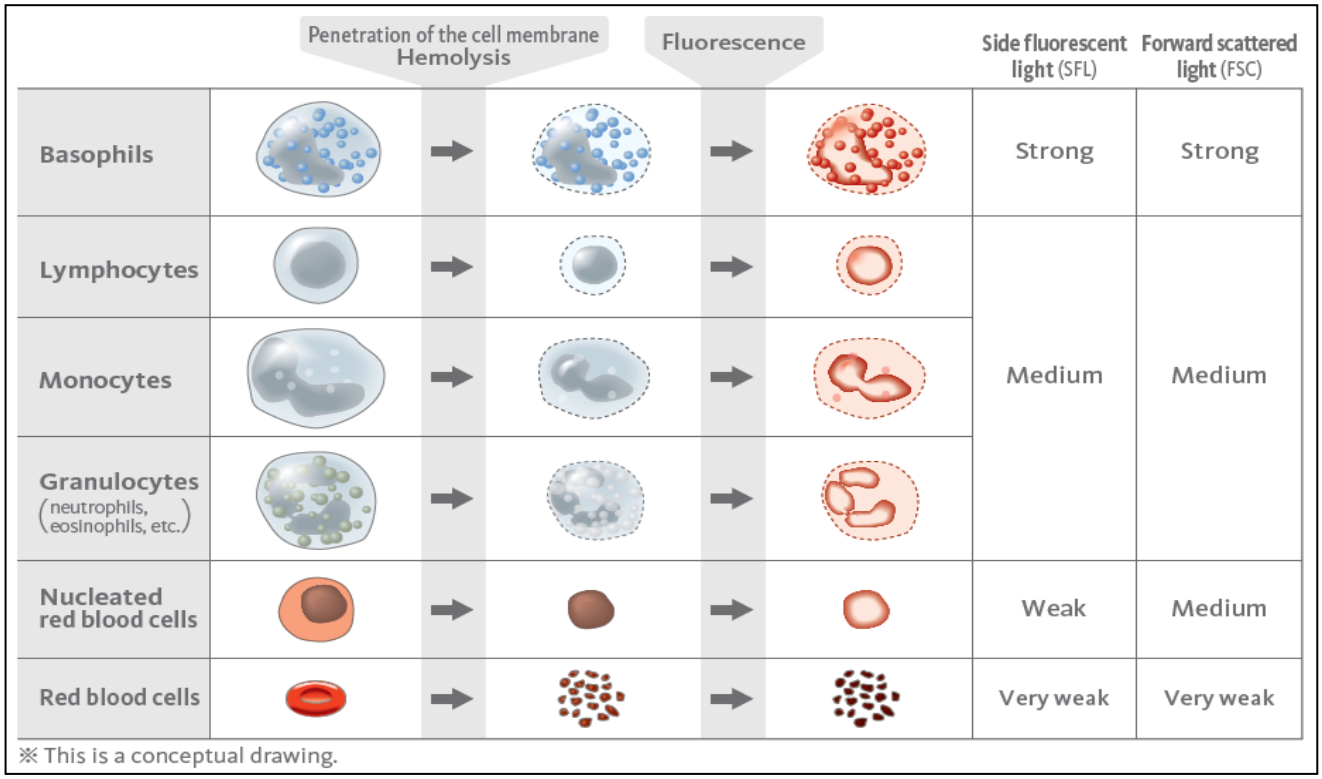
Fluorocell WNR

Fluorocell WNR is used to stain the nucleated cells following the lysing reaction described above. Nuclear and granular contents of the cells are stained allowing the cells to be differentiated due to their staining intensity and size.



Scattergram

Following reagent reactions and the analysis of the cells using fluorescence flow cytometry, the different cell populations are placed on the WNR Channel scattergram based on their forward scattered light (FSC) and side fluorescent light (FSL)

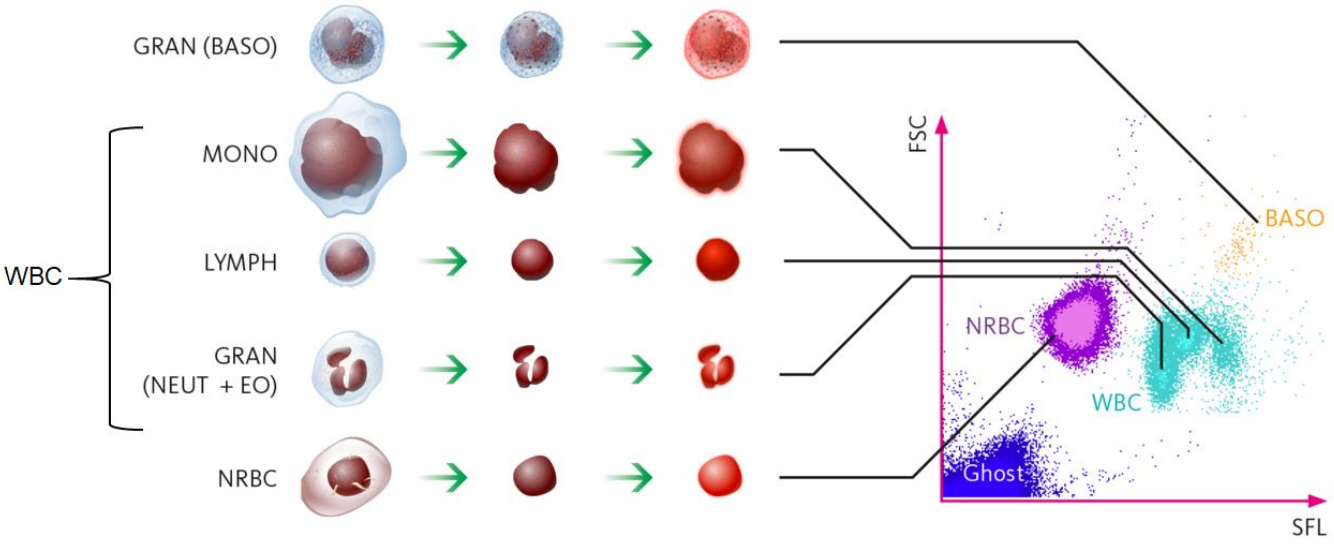


Populations:

Basophils: The basophil population is not affected by the shrinking action of the Lysercell WNR and are therefore maintained in a near native state, making them the largest cell, resulting in a strong FSC and due to the large amounts of RNA/DNA and organelle content they also display a strong SFL.

NRBC: The NRBC display a medium FSC and weak SFL due to their reduced susceptibility to the stain.

Non-Basophilic WBC population: This includes monocytes, lymphocytes, neutrophils and eosinophils. The non-basophilic WBC population are shrunk by the action of Lysercell WBC and are therefore a similar size to the NRBC nuclei, thus WBC's display medium FSC, however, WBC's have more RNA/DNA than NRBCs, therefore, display medium SFL.



The WNR Channel is used to report the following parameters:

- Basophil (BA-N) # and %
- Total WBC count (WBC-N): The total WBC count is calculated by taking the sum of the basophil population and the non-basophil WBC population.
- NRBC count # and %: A NRBC count is reported on all full blood count samples processed on the XN-Series analysers.

The WNR Channel also produces the following research parameters*:

- Total Nucleated Count (TNC-N) # and %: The total nucleated cell count (WBC + NRBC) calculated from the WNR channel.

* This list is not exhaustive.

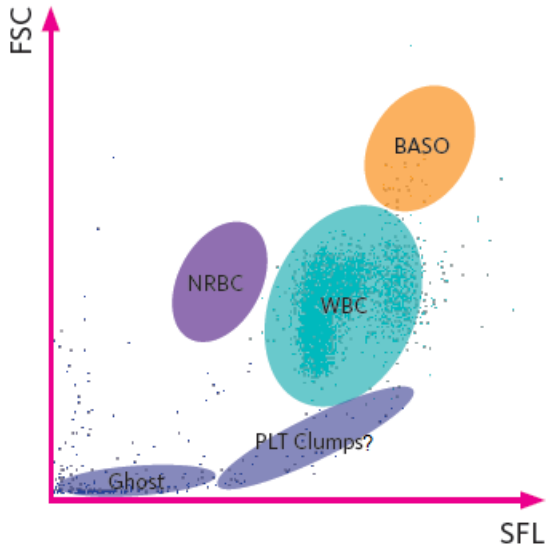
The XN-Series analysers calculate a ratio between the WBC-N count, calculated from the WNR channel, and the WBC-D count, calculated from the WDF channel, to ensure both channels are generating similar results. If the results obtained from both channels differ considerably, a [Review] flag will be generated.

Flagging Regions

Flags generated in the WNR Channel include:

- PLT Clumps?
- Giant PLTs?*
- WBC Abn Scattergram*

*** NOTE**: These flags do not refer to a specific flagging area on the scattergram. They are triggered by abnormal activity in the scattergram or through reviewing multiple scattergrams in conjunction with each other.



added value
XN-DIFF

XN-DIFF (WDF) Channel

The XN-DIFF (WDF) is a standard channel on both XN-10 and XN-20 analysers. Fluorescence flow cytometry within the XN-DIFF (WDF) Channel is used to differentiate neutrophils, lymphocytes, monocytes, eosinophils and immature granulocytes (IGs), using CellPack DCL/DST (as an isotonic diluent), Lysercell WDF & Fluorocell WDF.

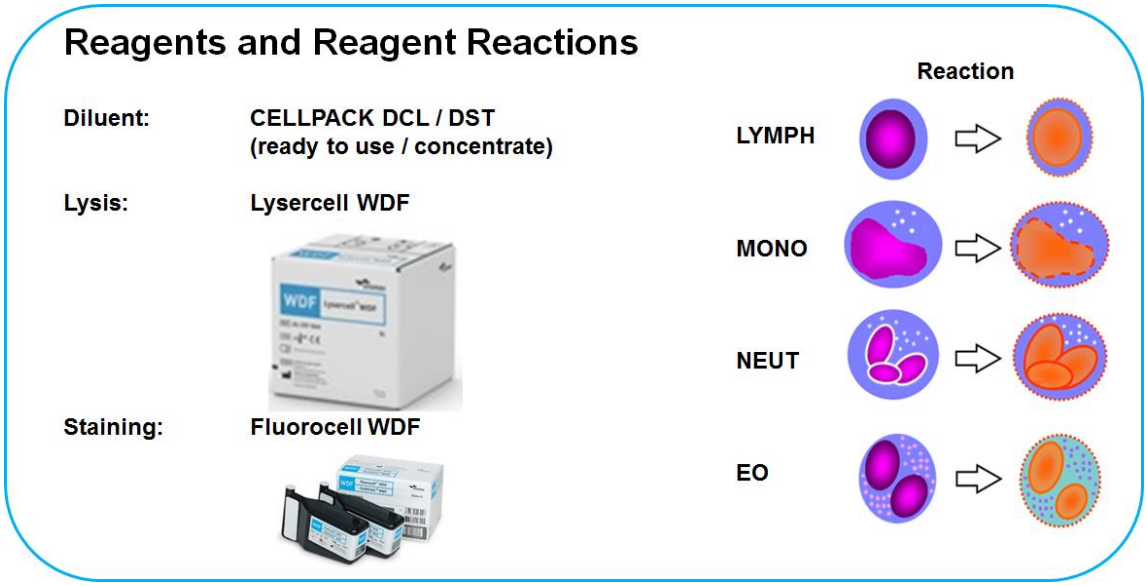
Reagents

Lysercell WDF

The Lysercell WDF reagent haemolyses red blood cells and the membranes of WBCs are made permeable to allow the Fluorocell WDF to enter the cells.

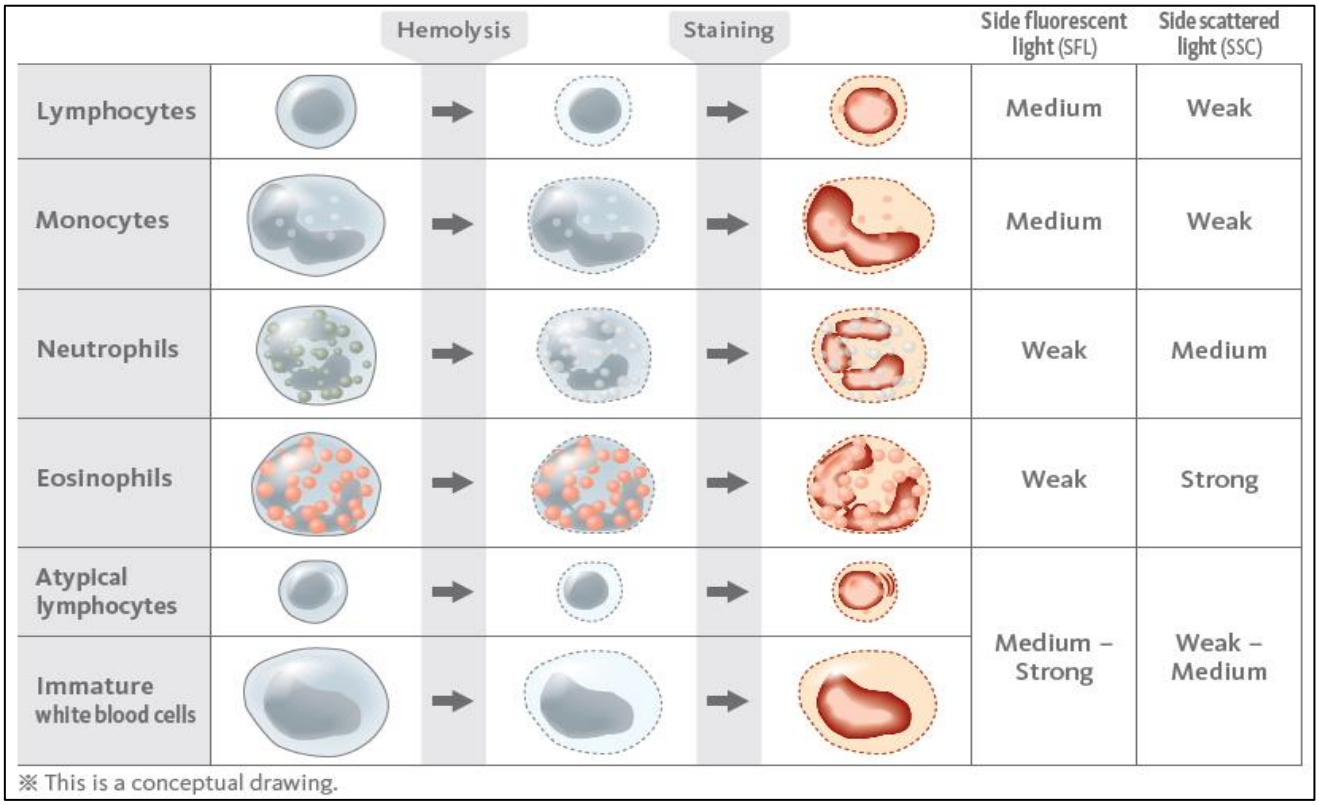
Fluorocell WDF

Fluorocell WDF is used to stain the nucleated cells following the lysing reaction described above. Nuclear and granular contents of the cells are stained allowing the cells to be differentiated due to their staining intensity and internal cellular complexity



Scattergram

Following reagent reactions and the analysis of the cells using fluorescence flow cytometry, the different cell populations are placed on the WDF Channel scattergram based on their side scattered light (SSC) and side fluorescent light (SFL).



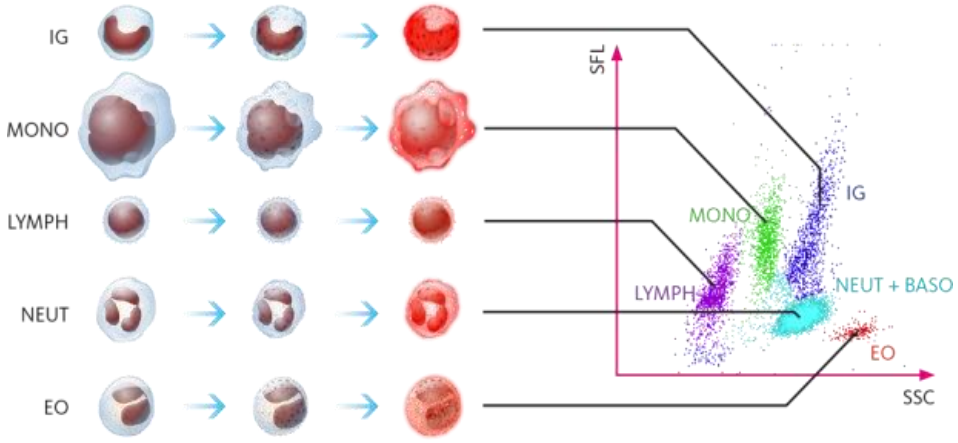
Populations:

Lymphocytes: Lymphocytes display weak SSC due to their low complexity. Lymphocytes also contain a relatively small amount of RNA/DNA and organelle content giving them medium SFL.

Monocytes: Monocytes display a slightly stronger SSC than lymphocytes due to their increased granularity and more complex shape of the nucleus. They show medium SFL due to their RNA/DNA and organelle content.

Neutrophils and Eosinophils: Granulocytes are separated based on increasing granular staining and complexity. Neutrophils display medium SSC due to their complexity and weak SFL whereas eosinophils being the most complex mature cell display strong SSC and weak SFL thus distinguishing the two populations.

Immature Granulocytes: The immature granulocytes (IGs) count is composed of promyelocytes, myelocytes and metamyelocytes. IGs display weak to medium SCC depending on the level of maturity of the cell and medium to strong SFL depending of the level of immaturity of the cell.



The WDF Channel is used to report the following parameters:

- Lymphocyte # and %
- Monocyte # and %
- Neutrophil # and %: The neutrophil count is calculated by subtracting the basophil count obtained in the WNR channel (BA-N) from the NEUT + BASO cloud in the WDF Channel. The NEUT + BASO cloud is comprised of the neutrophil count and the BA-D count.
- Eosinophil # and %
- Immature Granulocytes (IGs) # and %: The IG count is composed of promyelocytes, myelocytes and metamyelocytes and is reported on all full blood count samples processed on the XN-Series analysers

The WDF Channel also produces the following research parameters*:

- Total Nucleated Count (TNC-D) # and %: The total nucleated cell count (WBC count + nucleated RBC count) calculated from the WDF channel.
- Total White Cell Count (WBC-D) # and %: The total WBC count calculated from the WDF channel by adding together the neutrophil, lymphocyte, monocyte, eosinophil and basophil populations. NOTE: This is **NOT** reported.
- Basophil (BA-D) # and %: The basophil count calculated from the WDF channel.

* This list is not exhaustive.

The XN-Series analysers calculate a ratio between the WBC-D count, calculated from the WDF channel, and the WBC-N count, calculated from the WNR channel, to ensure both channels are generating similar results. The analyser also compares the basophil count calculated in the WNR channel (BA-N) to the basophil count calculated in the WDF channel (BA-D), to ensure both channels are generating a similar result. If the results obtained from both channels differ considerably, a [Review] flag will be generated.

Low White Cell Mode (LW)

When analysing a sample in low white cell mode (LW) the count time of the WDF channel is set to 3 times that of whole blood mode to increase the WBC measurement accuracy.

The white cell parameters produced in low white cell mode (LW) are generated from the WDF channel, using the same reagents and include:

- Total WBC: The total WBC count is calculated by taking the sum of white cell populations below.
- Lymphocyte # and %
- Monocyte # and %
- Neutrophil # and %
- Eosinophil # and %
- Basophil # and %
- Immature Granulocytes (IGs) # and %

Flagging Regions

The triggering of suspect flags in the WDF channel is based on an algorithm taking the following factors into account:

- A special shape-recognition algorithm termed AFLAS.
- Forward scattered light levels (FSC), side fluorescent light levels (SFL) and side scattered light levels (SSC), which reflect the functional state of the cells
- Cell counts.
- Presence of cells in defined areas (gates) and the ratios of these cell counts.

AFLAS

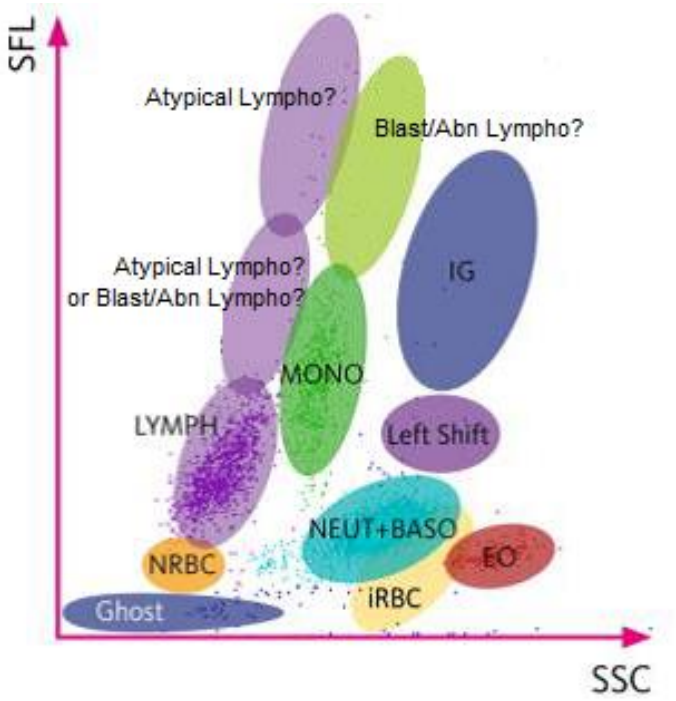
'Adaptive Flagging Algorithm based on Shape recognition' (AFLAS) is based on 6 different parameters used to describe a cell population (cloud):

- Size of the cloud (Total size of the population)
- Length of the cloud (Longitudinal distance)
- Width of the cloud (Transversal distance)
- Position (Centre of the cloud)
- Momentum (Shape of the cloud)
- Angle (Direction of the longitudinal axes)

Flags generated in the WDF Channel include:

- Left Shift?
- Atypical Lympho?
- Blast/Abn Lympho?
- PLT Clumps?*
- WBC Abn Scattergram*
- iRBC? Flag

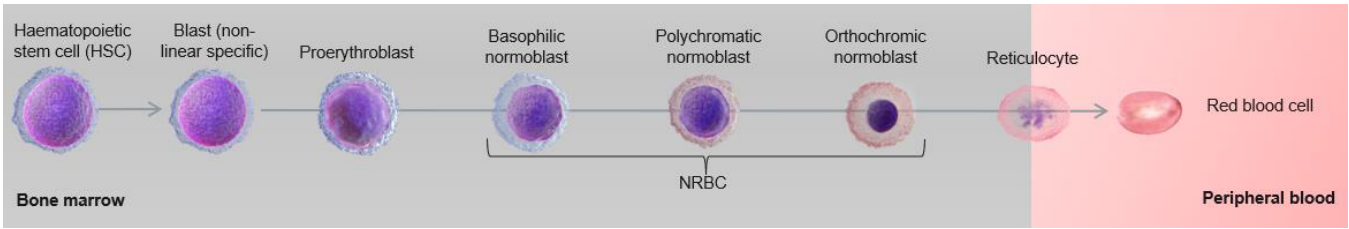
*** NOTE:** These flags do not refer to a specific flagging area on the scattergram. They are triggered by abnormal activity in the scattergram or through reviewing multiple scattergrams in conjunction with each other.



added value
RET

RET Channel

The XN-RET channel is a standard channel on the XN-20 analyser but is optional for the XN-10 analyser. Fluorescence flow cytometry within the RET Channel is used to produce diagnostic reticulocyte parameters (including information regarding reticulocyte maturation), as well as providing additional information about the quality of the newly formed RBCs following erythropoiesis (see image below). The RET channel also provides an alternative technology for producing a platelet count (PLT-O), eliminating some of the issues associated with platelet and RBC counts obtained by sheath flow (DC) detection, such as, large platelets being counted as RBCs and therefore, producing a falsely increased RBC count, leading to a falsely decreased PLT count or small RBCs causing falsely increased PLT counts, leading to falsely decreased RBC counts.



The XN-RET channel uses CellPack DCL/DST (as an isotonic diluent), CellPack DFL and Fluorocell RET.

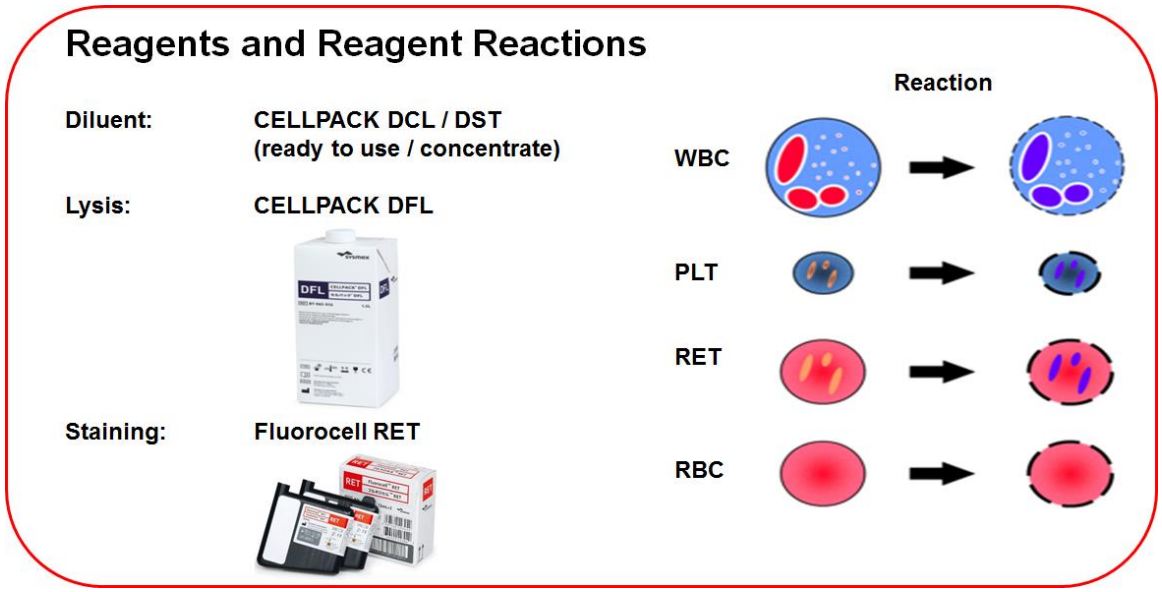
Reagents

CellPack DFL

CellPack DFL is a diluent which contains a surfactant that causes the cell membranes of WBCs, RBCs, reticulocytes and platelets to become permeable.

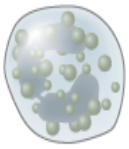
Fluorocell RET

Fluorocell RET can enter the cells following the reaction with CellPack DFL and stain the nuclear and granular contents of the cells. As the reticulocytes contain RNA these will be stained, unlike the mature RBCs which do not contain RNA and therefore show limited staining due small amount of organelle content remaining. Granular contents of platelets are also stained by Fluorocell RET allowing an optical platelet count to be achieved (PLT-O).



Scattergram

Following reagent reactions and the analysis of the cells using fluorescence flow cytometry, the different cell populations are placed on the RET Channel scattergram based on their forward scattered light (FSC) and side fluorescent light (SFL).

	Staining	Side fluorescent light (SFL)	Forward scattered light (FSC)
White blood cells	 → 	Strong	Strong
Reticulocytes	 → 	Medium	Strong
Red blood cells	 → 	Weak	Strong

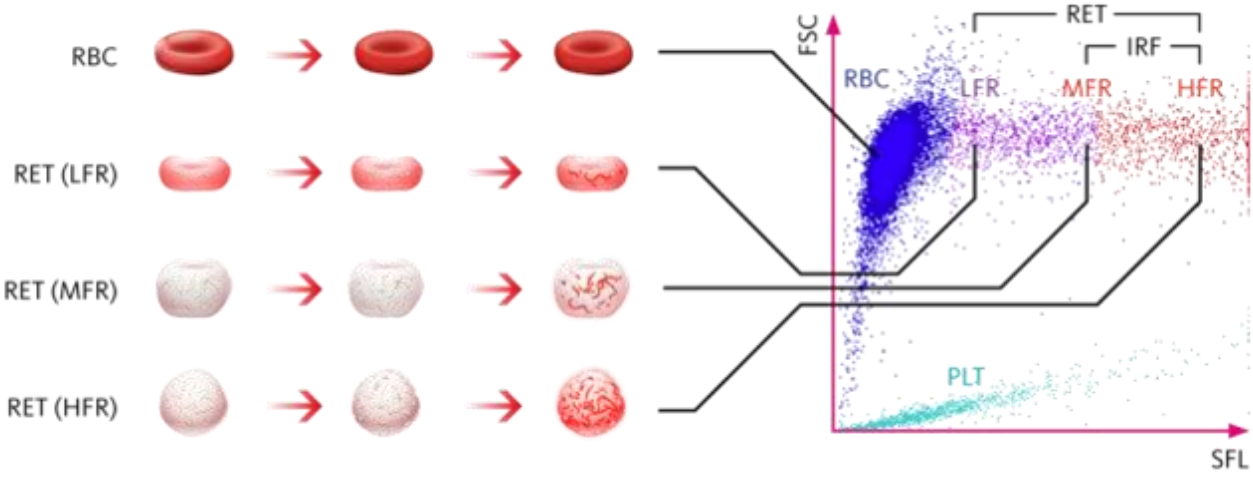
※ This is a conceptual drawing.

Populations:

RBC: Mature RBC's display weak SFL due to the lack of RNA and DNA, and display strong FSC due to them being large in size. The mature RBC population may move on the FSC axis depending on the size of the red cell.

Reticulocytes: Reticulocytes display medium SFL, due to their RNA content, and strong FSC. The reticulocytes are further broken down into low, medium and high fluorescing populations (LFR, MFR and HFR respectively), due to their increasing RNA content. Together this gives the total reticulocyte count.

Optical Platelet Count: Platelets display weak FSC due to their small size and weak SFL due to lack of DNA and limited amount of RNA depending on platelet age.



The XN-RET Channel is used to report the following parameters:

- Reticulocyte count # and %
- Optical Platelet count (PLT-O)
- The three stages of reticulocyte maturation:
  - High Fluorescence Ratio (HFR) – 'immature' reticulocytes
  - Medium Fluorescence Ratio (MFR) – 'semi-mature' reticulocytes
  - Low Fluorescence Ratio (LFR) – 'mature' reticulocytes
- Immature Reticulocyte Fraction (IRF): The IRF is calculated using the sum of the HFR and MFR and can be used as an indicator for effective erythropoiesis.
- RET-H_e: RET-H_e is the average haemoglobin concentration of the reticulocytes and can be used to monitor responses to therapy.

The RET Channel also produces the following research parameters*:

- RBC-O: RBC Count calculated from the RET channel.
- HGB-O: Haemoglobin concentration calculated from the RET channel.
- RET-UPP: The count in the upper area of the RET scattergram.
- Fragmented Red Cells (FRC) # and %: The absolute count calculated from the count in a specific area below the RBC area in the RET scattergram.

* This list is not exhaustive.

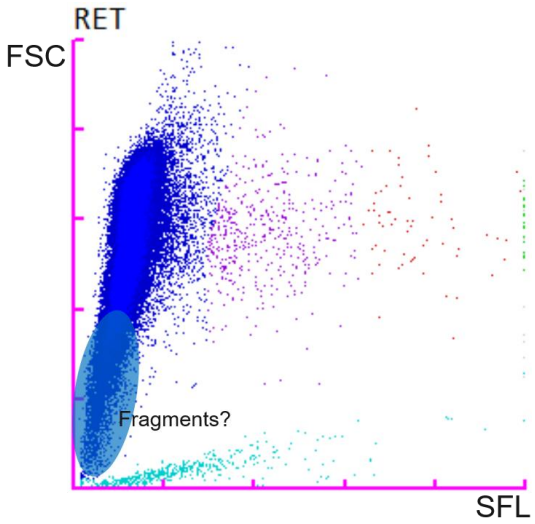
The XN-Series analysers calculate a ratio between the RBC-O count, calculated from the RET channel, and the RBC count, calculated via sheath flow (DC) detection, to ensure both channels are generating similar results. The analyser also compares the platelet count (PLT-O), calculated in the RET channel, to the platelet count calculated via sheath flow (DC) detection, to ensure both channels are generating similar results. If the results obtained from both channels differ considerably, a [Review] flag will be generated.

Flagging Regions

Flags generated in the XN-RET Channel include:

- Fragments?
- RET Abn Scattergram*

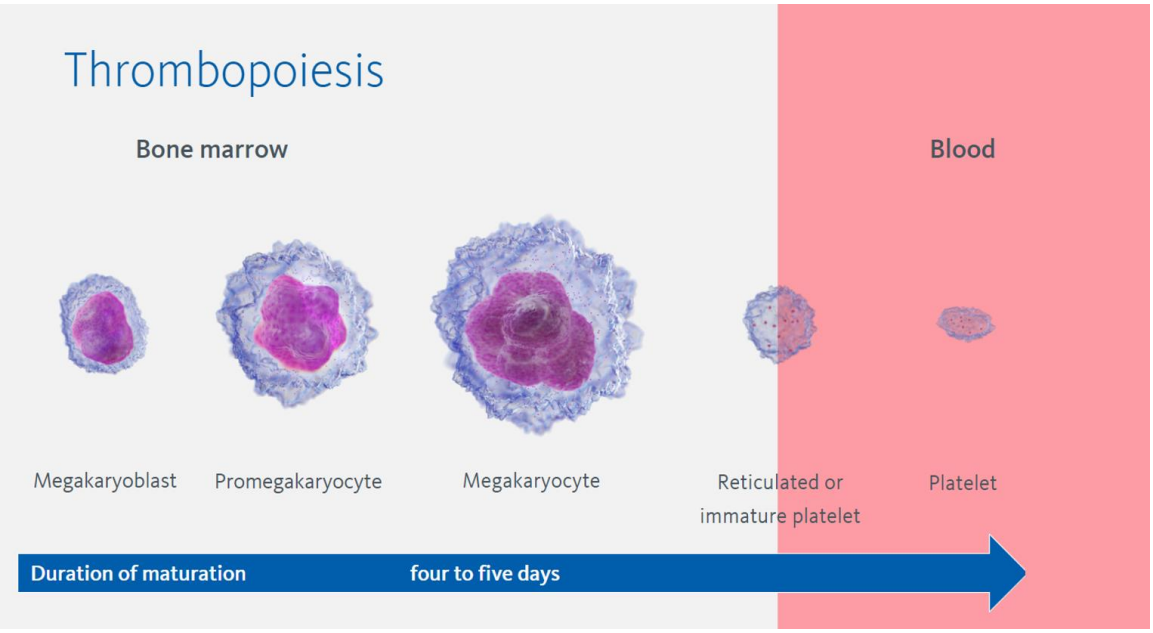
*** NOTE:** These flags do not refer to a specific flagging area on the scattergram. They are triggered by abnormal activity in the scattergram or through reviewing multiple scattergrams in conjunction with each other.



added value
PLT-F

PLT-F Channel

The PLT-F channel is an optional channel for both the XN-10 and XN-20 analysers. Specialised fluorescence flow cytometry within the PLT-F channel is used to generate an accurate and precise platelet count, referred to as the fluorescent platelet count (PLT-F), which is comparable to the CD41/CD61 reference method. This channel can give an accurate count even in platelet disorders, whether the disorder is due to platelet number or platelet size. The PLT-F channel analyses a 5-fold larger sample volume of the aspirated sample compared to the Sheath Flow (DC) Detection measurement, which results in the high measurement accuracy in the low concentration range. The PLT-F channel also provides information about immature platelets in the form of the immature platelet fraction (IPF).



During thrombopoiesis, immature platelets (seen in the diagram above) are produced from the budding of the megakaryocyte, following which they mature to become a platelet. In comparison to mature platelets, immature platelets are slightly larger in size and contain more RNA, which is morphologically indistinguishable. Immature platelets have a short lifespan, approximately 1-2 days, following which they shrink and lose some of their RNA content, becoming a mature platelet.

The IPF can be used to:

1. Indicate whether the cause of thrombocytopenia is due to destruction or production issues:
    a. In platelet destruction, there would be a high IPF as the bone marrow is producing the platelets before they are destroyed
    b. In production issues, as the bone marrow is not producing any platelets, then the IPF would be low.
2. Assess bone marrow recovery

The PLT-F channel uses CellPack DCL/DST (as an isotonic diluent), CellPack DFL and Fluorocell PLT.

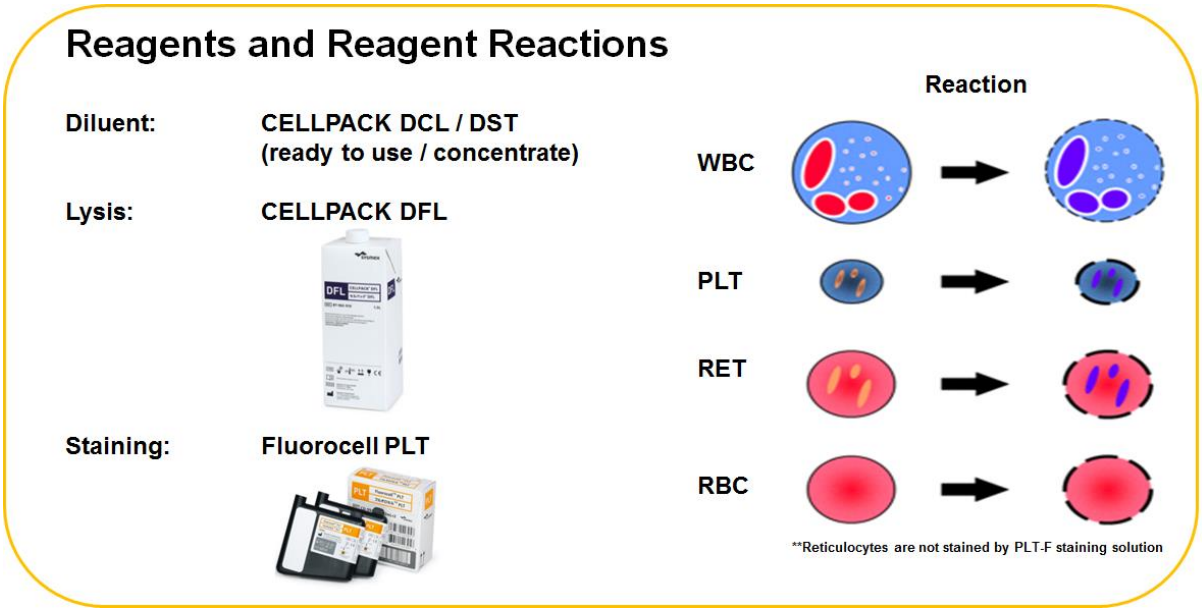
Reagents

CellPack DFL

CellPack DFL is a diluent which contains a surfactant that causes the cell membranes of the platelets to become permeable.

Fluorocell PLT

Fluorocell PLT can enter the cells following the reaction with CellPack DFL and specifically stains the RNA within the platelets. The fluorescence marker found in the Fluorocell PLT specifically labels platelets and non-specifically labels RBCs and WBCs, however, the analyser can differentiate between the different populations, which minimises interferences and is a reason for the extremely good correlation with the CD41/CD61 flow cytometry method.



Scattergram

Following reagent reactions and the analysis of the cells using fluorescence flow cytometry, the different cell populations are placed on the PLT-F Channel scattergram based on their forward scattered light (FSC) and side fluorescent light (SFL).

	Staining	Side fluorescent light (SFL)	Forward scattered light (FSC)
Red blood cells		Weak – Medium	Strong
Platelets		Weak – Medium	Weak
IPF (Immature platelet fraction)		Medium – Strong	Medium

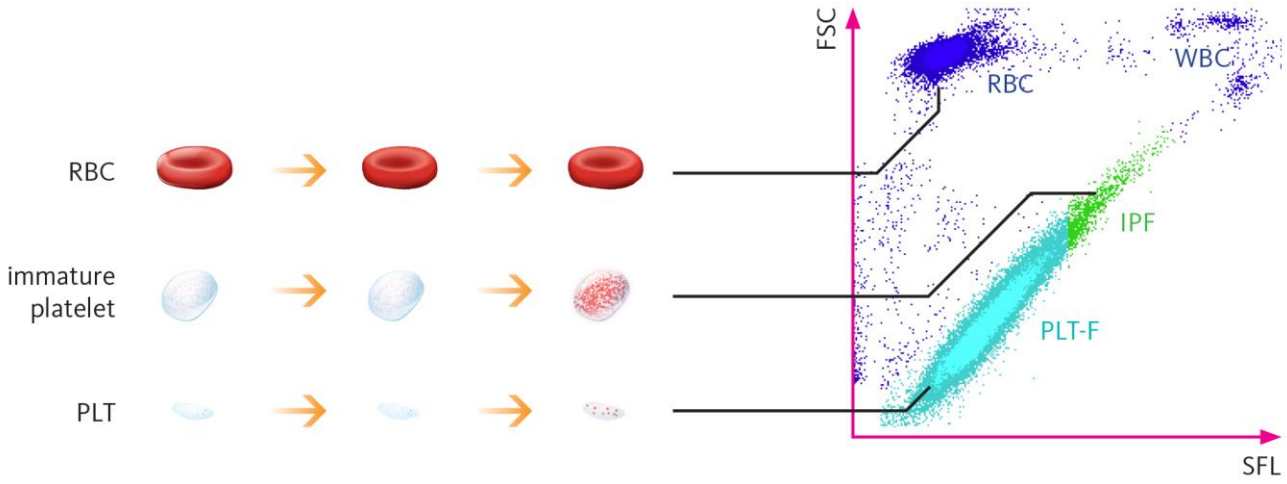
※ This is a conceptual drawing.

Populations:

Platelets: Platelets display weak FSC due to their small size and weak to medium SFL due to their RNA content. As immature platelets are larger than mature forms and contain more RNA, they display a stronger FSC and SFL, allowing an immature platelet fraction (IPF) to be calculated.

RBC: RBC's display a strong FSC, due to their size and weak SFL due to their lack of DNA/RNA and residual organelle content.

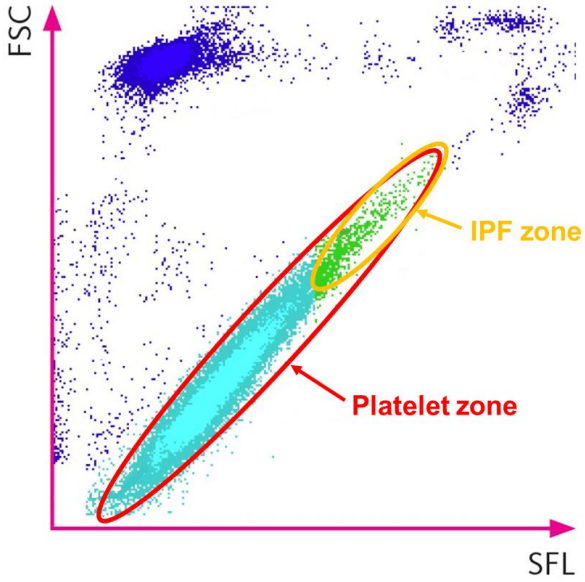
WBC: WBC's display a strong FSC, due to their large size and strong SFL due to their nucleic acid content.



The PLT-F Channel is used to report the following parameters:

- Fluorescent Platelet count (PLT-F) – calculated from the platelet zone
- IPF # and %: The IPF % is relative to the platelet count and is calculated using the equation below.

$$\text{IPF \%} = \frac{\text{Particle count in IPF zone}}{\text{Particle count in the platelet zone}} \times 100$$



The XN-Series analysers calculate a ratio between the RBC-O count, calculated from the RET channel, and the RBC count, calculated via sheath flow (DC) detection, to ensure both channels are generating similar results. If the results obtained from both channels differ considerably, a [Review] flag will be generated.

Flagging Regions

Flags generated in the PLT-F Channel include:

- PLT Clumps?*
- PLT Abn Scattergram*

*** NOTE:** These flags do not refer to a specific flagging area on the scattergram. They are triggered by abnormal activity in the scattergram or through reviewing multiple scattergrams in conjunction with each other.

WPC Channel

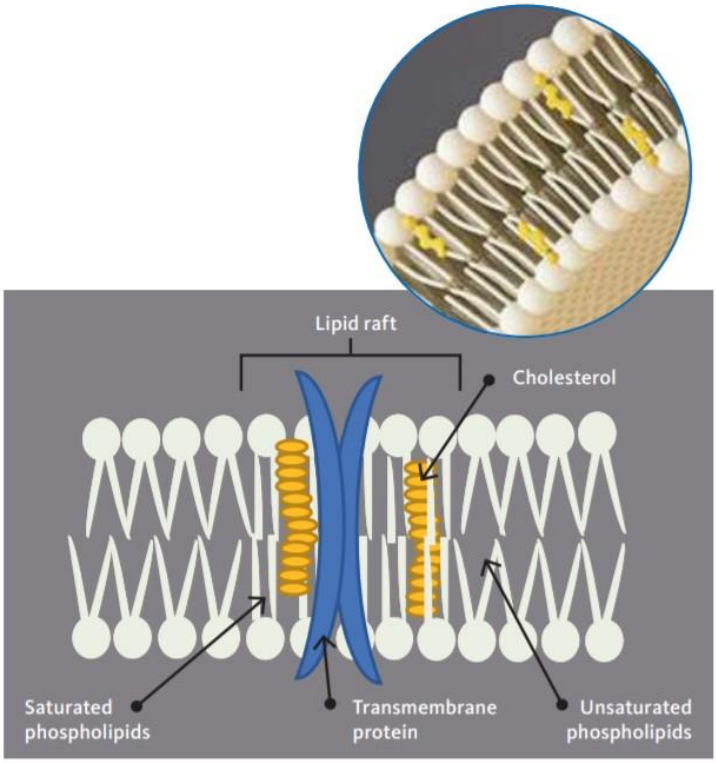
The WPC (White Pathological and Precursor Cell) channel is a standard channel on an XN-20 analyser, but due to the requirement for additional hardware it cannot be added on to an XN-10 analyser. Fluorescence flow cytometry within the WPC channel allows for enhanced flagging information to be generated regarding immature WBCs i.e. blasts and abnormal lymphocytes. The WPC channel further classify samples initially flagged positive for 'Blasts/Abn Lympho?' by the XN-DIFF channel.

The reagent reactions in the WPC channel depend specifically on the composition of the membrane lipids.

Cell membranes are constructed of a phospholipid bilayer and within this bilayer there are micro domains referred to as lipid rafts.

Lipid rafts are composed of transmembrane proteins, cholesterol molecules and glycolipids, and they play important roles in protein trafficking and cellular signaling. Lipid rafts are more ordered and tightly packed than the surrounding membrane bilayer, but float freely in this bilayer.

White blood cells have different membrane compositions depending on their maturity, function and activation status. Also lipid rafts vary between resting mature cells, activated cells (e.g. T lymphocytes) and immature cells, which means they are affected differently by the reagents used within the WPC channel, ultimately enabling the analyser to differentiate between them.



The WPC channel uses CellPack DCL/DST (as an isotonic diluent), Lysercell WPC and Fluorocell WPC.

Reagents

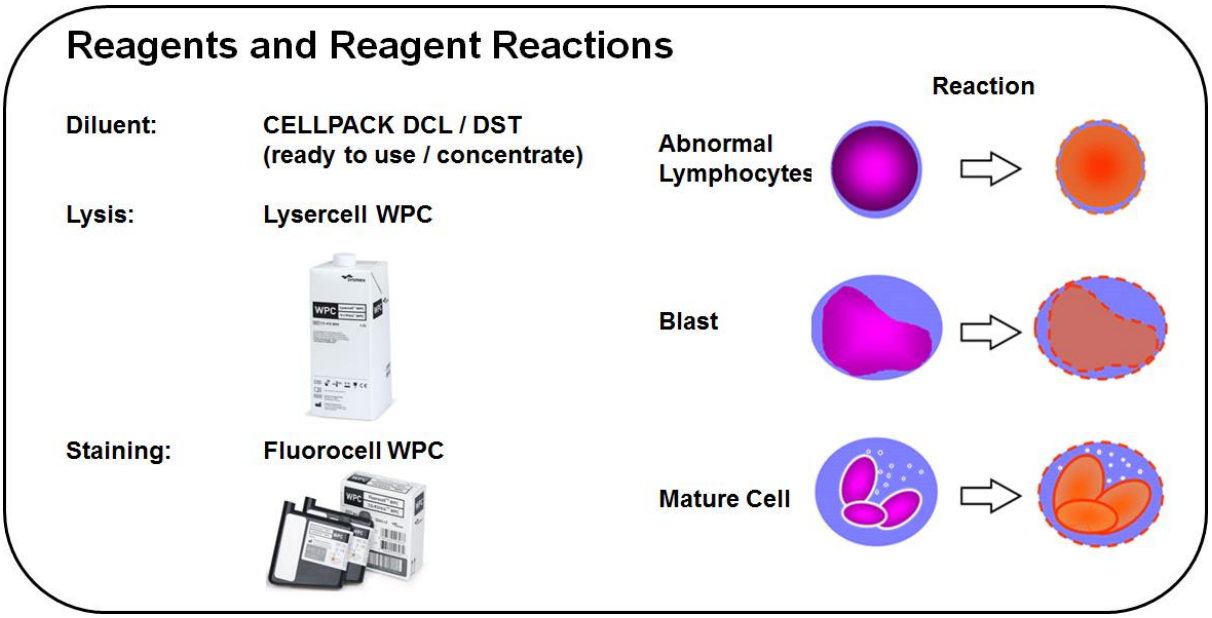
Lysercell WPC

The Lysercell WPC reagent lyses the RBCs and acts upon the lipid rafts within the cell membrane of the WBCs. The loss of the lipid rafts from the cell membrane effects the permeabilisation of the cell.

In addition, the higher the degree of membrane perforation by Lysercell WPC lysing, the more cellular content leaks through the pores. This results in a smaller cell size and more Fluorocell WPC entering the cell.

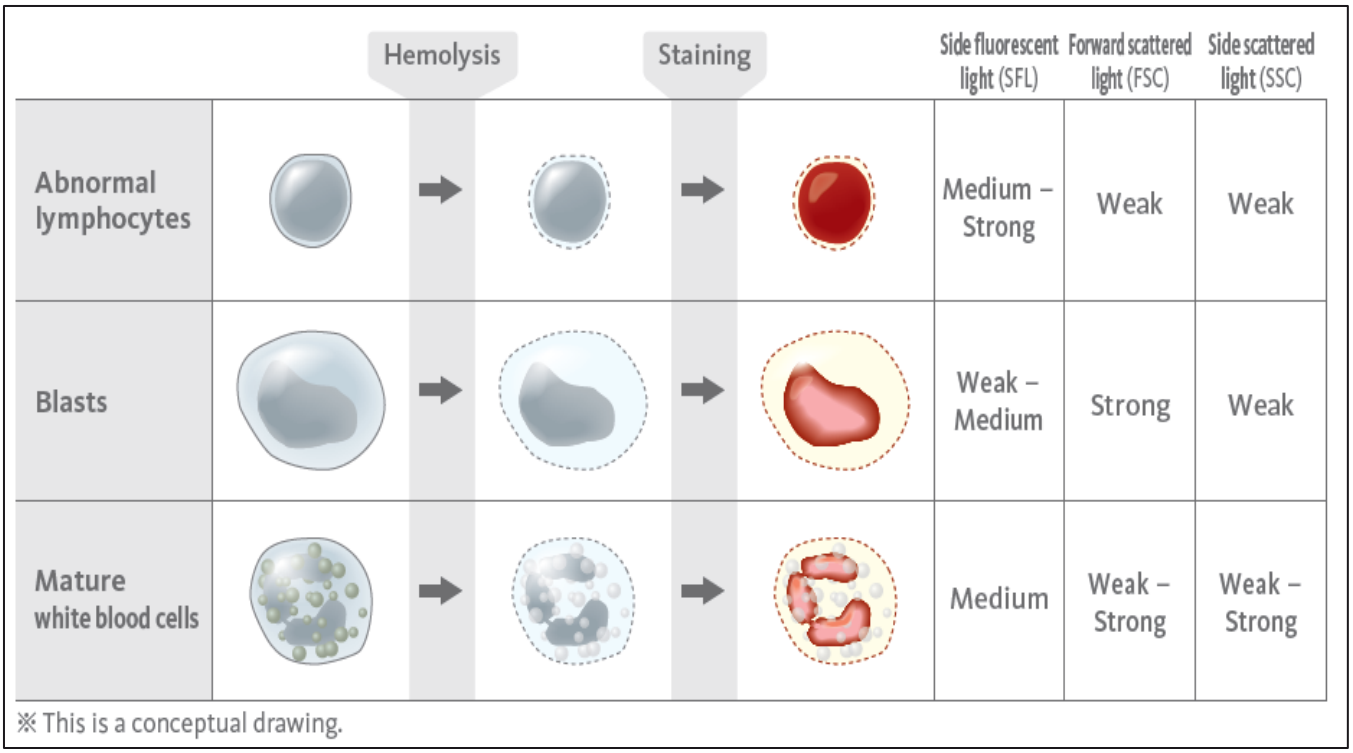
Fluorocell WPC

The Fluorocell WPC reagent can enter the cells following the reaction with Lysercell WPC and as it has a higher polymethine concentration than the Fluorocell WDF reagent, it will stain mainly the DNA within the nucleus of the cell instead of just the cytoplasmic RNA.



Scattergram

Following reagent reactions and the analysis of the cells using fluorescence flow cytometry, the different cell populations are placed on the WPC Channel scattergram based on their forward scattered light (FSC), side scattered light (SSC) and side fluorescent light (SFL).



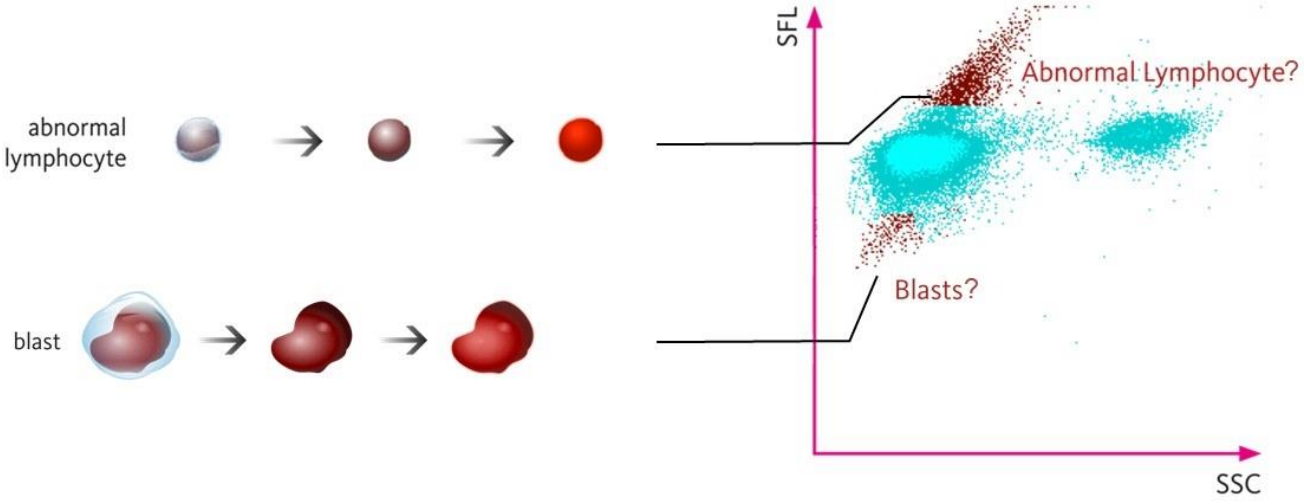
Populations:

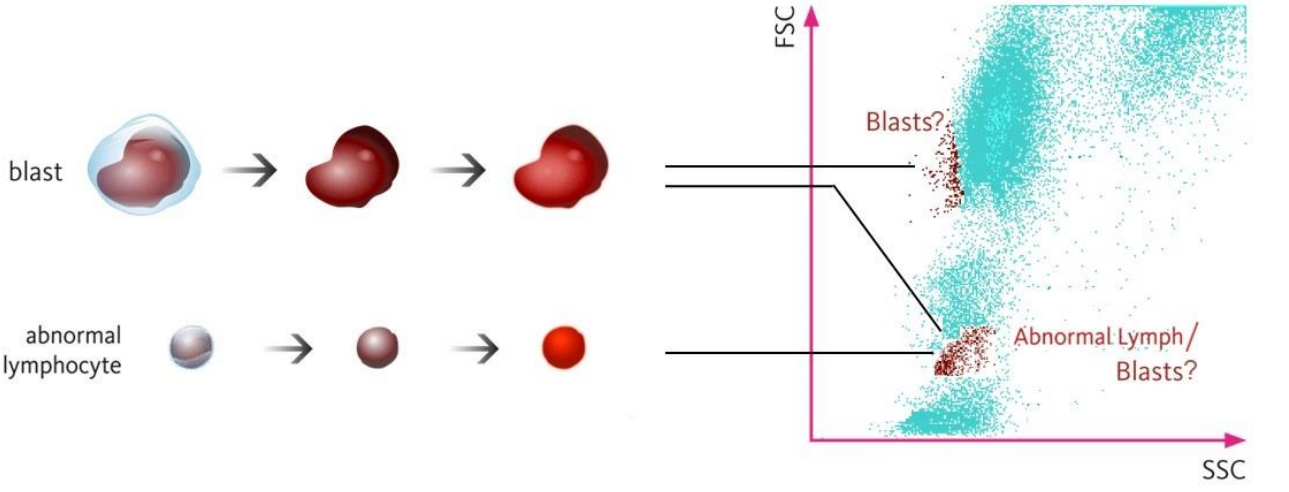
The WPC channel is used mainly for flagging purposes and produces flags for blasts (Blasts?) and abnormal lymphocytes (Abn Lympho?) or alternatively removing the Abn Lymph/Blast? flag generated in the WDF Channel.

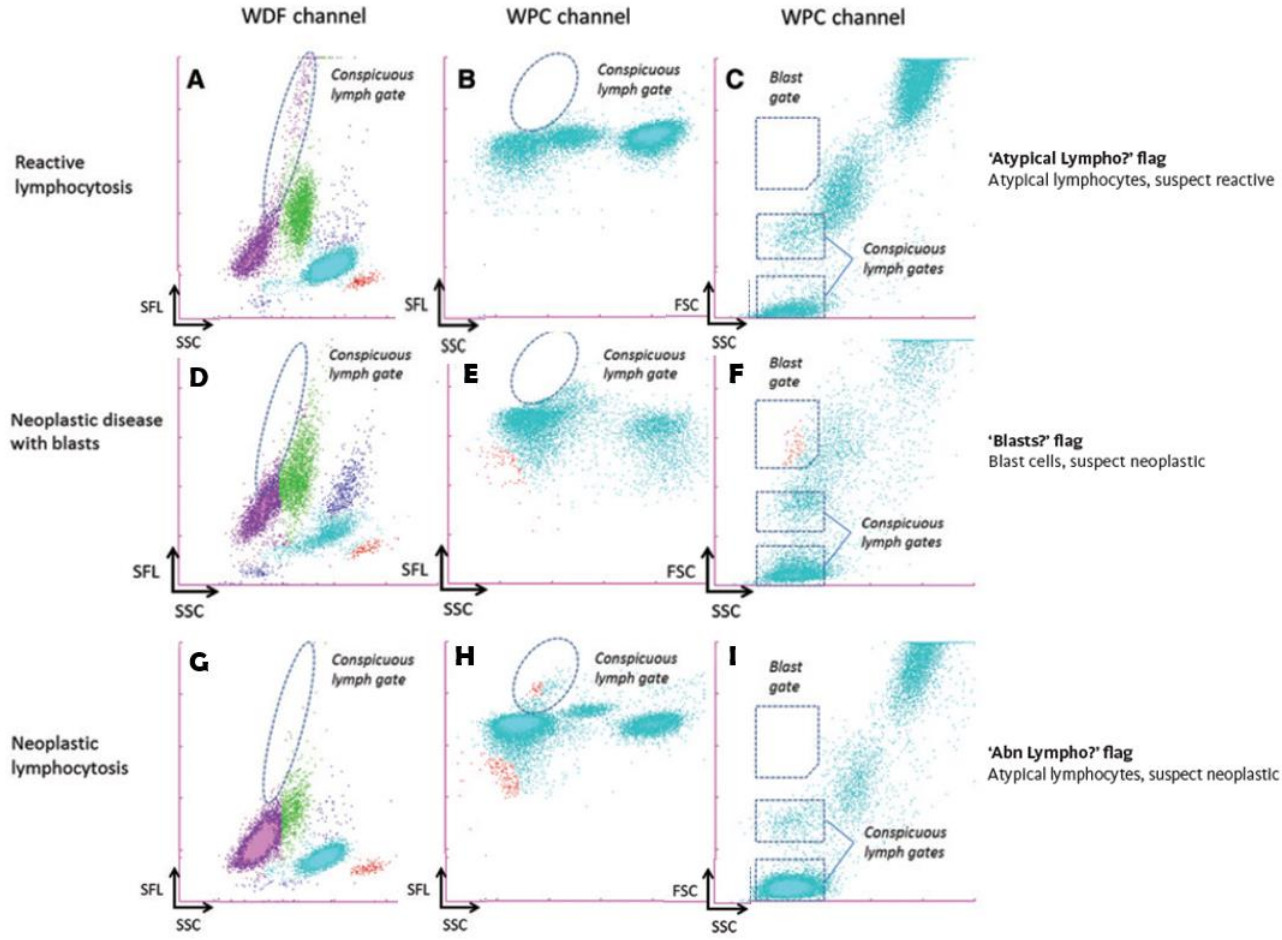
Blasts?: Due to their low membrane lipid composition, blasts are not permeated very strongly by the lysis reagent, which means less Fluorocell WPC can enter the cell, resulting in a relatively weak SFL. This also means less cellular content is lost, meaning the cell remains large and has a strong FSC. Blasts will show a weak SSC due to their lack of complexity.

Abn Lymphs?: Abnormal lymphocytes are more mature, meaning their membranes are more readily permeated, leading to cellular components leaking through the pores, resulting in a weak FSC. Additionally, more fluorescence marker can enter the cell and bind to nuclear DNA, which in turn leads to medium to strong SFL. Abnormal lymphocytes will show a weak SSC due to their lack of complexity.

Other WBCs: WBC's display a medium SFL and weak to strong SSC, depending on the WBC.







Following analysis in the WPC channel, the analyser uses combined intelligence, between the WDF channel scattergram and the WPC channel scattergrams, to differentiate between the different cell populations based on the activity generated.

The image above show examples of when the analyser generates the 'Atypical Lympho?' flag, 'Blast?' flag and 'Abn Lympho?' flag:

Atypical Lympho? Flag: The 'Atypical Lympho?' flag is derived from scattergrams A, B and C. Activity is seen in the 'Blast/Abn Lympho?' region of the WDF scattergram, no activity is seen in the 'Blast?' or 'Abn Lympho?' flagging regions on both WPC scattergrams, therefore, the 'Atypical Lympho?' flag is generated.

Blast? Flag: The 'Blast?' flag is derived from scattergrams D, E and F. Activity is seen in the 'Blast/Abn Lympho?' region of the WDF scattergram and activity is seen in both 'Blast?' flagging regions on both WPC scattergrams with no activity seen in the 'Abn Lympho?' flagging regions on the WPC scattergrams, therefore, the 'Blast?' flag is generated.

Abn Lympho? Flag: The 'Abn Lympho?' flag is derived from scattergrams G, H and I. Activity is seen in the 'Blast/Abn Lympho?' region of the WDF scattergram and activity is seen in the 'Abn Lympho?' and 'Blast?' flagging regions of scattergram H, however, because no activity is seen in the 'Blast?' flagging region of scattergram I only the 'Abn Lympho?' flag is generated.

The WPC Channel also produces the following research parameters*:

- WPC-P: The WBC count calculated from the WPC channel.
- TNC-P: The total nucleated cell count (WBC + NRBC) calculated from the WBC channel.

* This list is not exhaustive.

The XN-Series analysers calculate a ratio between the WPC-P count, calculated from the WPC channel, and the WBC-N) count, calculated in the WNR channel, to ensure both channels are generating similar results. If the results obtained from both channels differ considerably, a [Review] flag will be generated.

added value
XN-BF

BF (Body Fluid) Mode

The XN-BF mode is an optional application for both XN-10 and XN-20 analysers. The automated body fluid mode is dedicated to the analysis of CSF (cerebrospinal fluid), ascetic fluids, serous fluids, synovial fluids, drain fluids and continuous ambulatory peritoneal dialysate (CAPD). A special, prolonged counting sequence provides a precise count of nucleated cells, also in very low counting ranges, which are applicable for body fluids.

The mode utilises CellPack DCL/DST to perform the RBC count using sheath flow (DC) detection in the XN-CBC channel and CellPack DCL/DST (as an isotonic diluent), Lysercell WDF and Fluorocell WDF to perform the WBC count (mononuclear and polymorphonuclear cells) using fluorescence flow cytometry in the XN-DIFF channel.

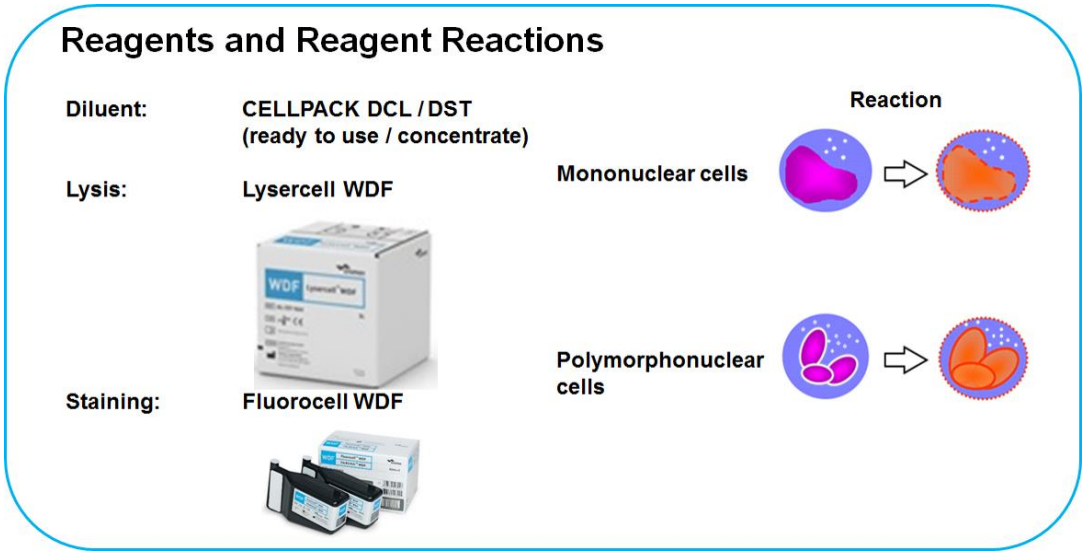
Reagents

Lysercell WDF

The Lysercell WDF reagent haemolyses red blood cells and the membranes of WBCs are made permeable to allow the Fluorocell WDF to enter the cells.

Fluorocell WDF

Fluorocell WDF is used to stain the nucleated cells following the lysing reaction described above. Nuclear and granular contents of the cells are stained allowing the cells to be differentiated due to their staining intensity and internal cellular complexity



Scattergram

Following reagent reactions and the analysis of the cells using fluorescence flow cytometry, the different cell populations are placed on the BF mode scattergram based on their side scattered light (SSC) and side fluorescent light (SFL).

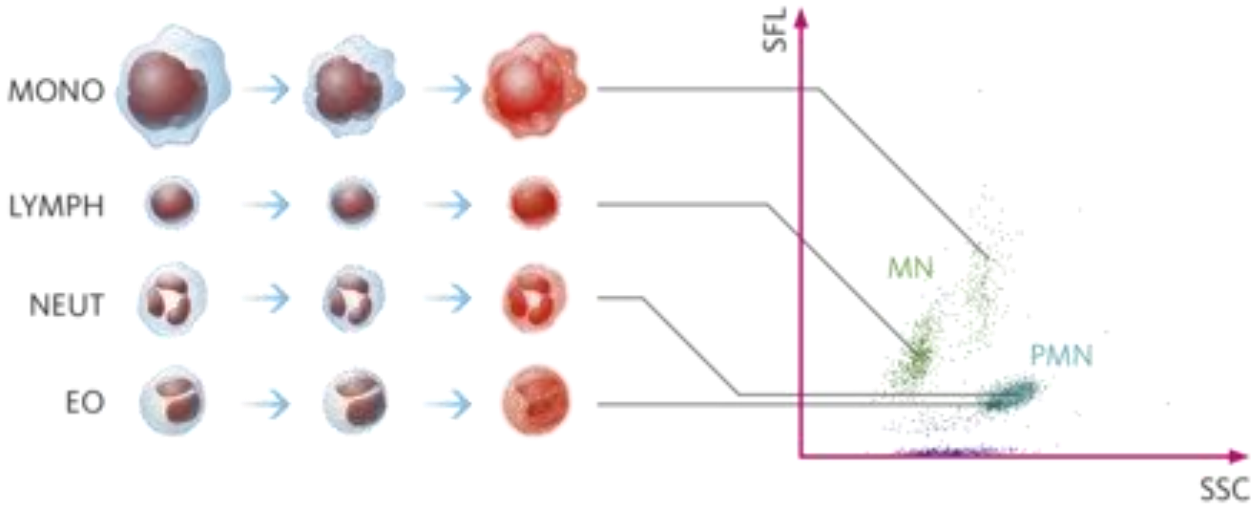
Populations:

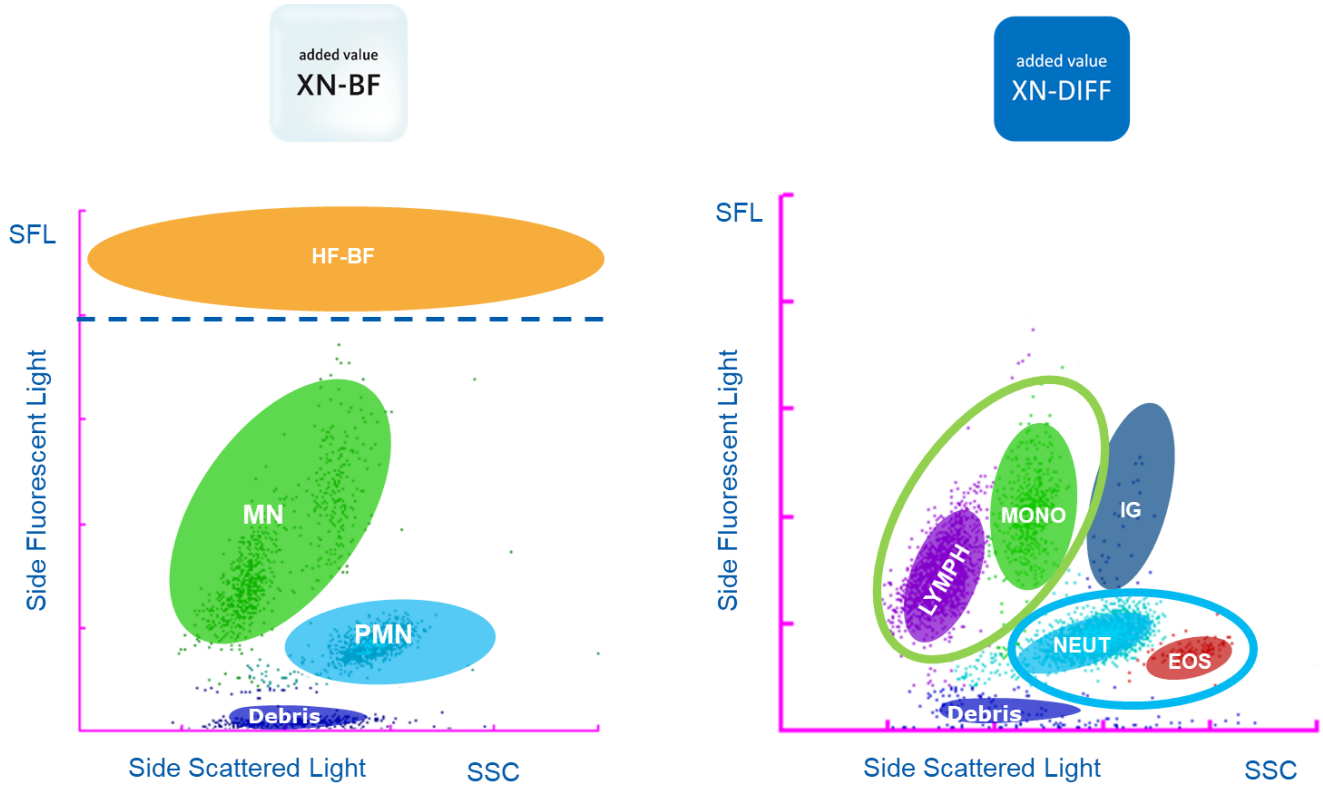
Mononuclear Cells (MN): Mononuclear cells (lymphocytes and monocytes) display medium to weak SFL and weak SSC.

Polymorphonuclear Cells (PMN): Polymorphonuclear cells (neutrophils, eosinophils and basophils) display weak SFL and strong SCC.

Research:

The High Fluorescence Body Fluid count (HF-BF) is based on their increased fluorescence when compared to the mature mononuclear and polymorphonuclear cells SFL. This population has been found to consist of epithelial cells.





The BF mode is used to report the following parameters:

- Mononuclear cells (MN) # and %
- Polymorphonuclear (PMN) # and %
- RBC
- WBC: The WBC count is a sum of the MN and PMN cells.
- Total Cells (TC-BF): The TC-BF is the sum of the WBC count and the HF-BF cells.

The BF mode also produces the following research parameters*:

- NE-BF # and %: The count in the neutrophil area of the WDF scattergram.
- LY-BF # and %: The count in the lymphocyte area of the WDF scattergram.
- MO-BF # and %: The count in the monocyte area of the WDF scattergram.
- EO-BF # and %: The count in the eosinophil area of the WDF scattergram.

* This list is not exhaustive.

Activities

XN-CBC (WNR) Channel Scattergram - Activity 1:

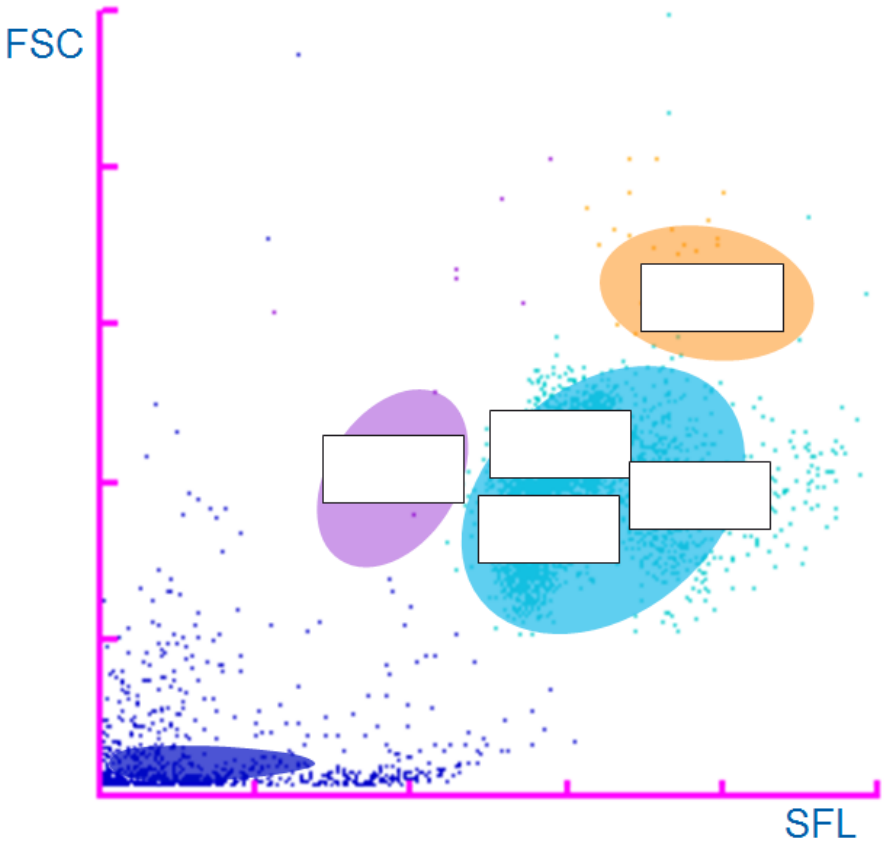
Using your knowledge of the XN-Series reagents and your understanding of fluorescence flow cytometry, please list the reagents used in this channel and label the scattergram below (including the axis).

Reagents used in this channel:

1. _____

2. _____

3. _____



XN-DIFF (WDF) Channel Scattergram - Activity 2:

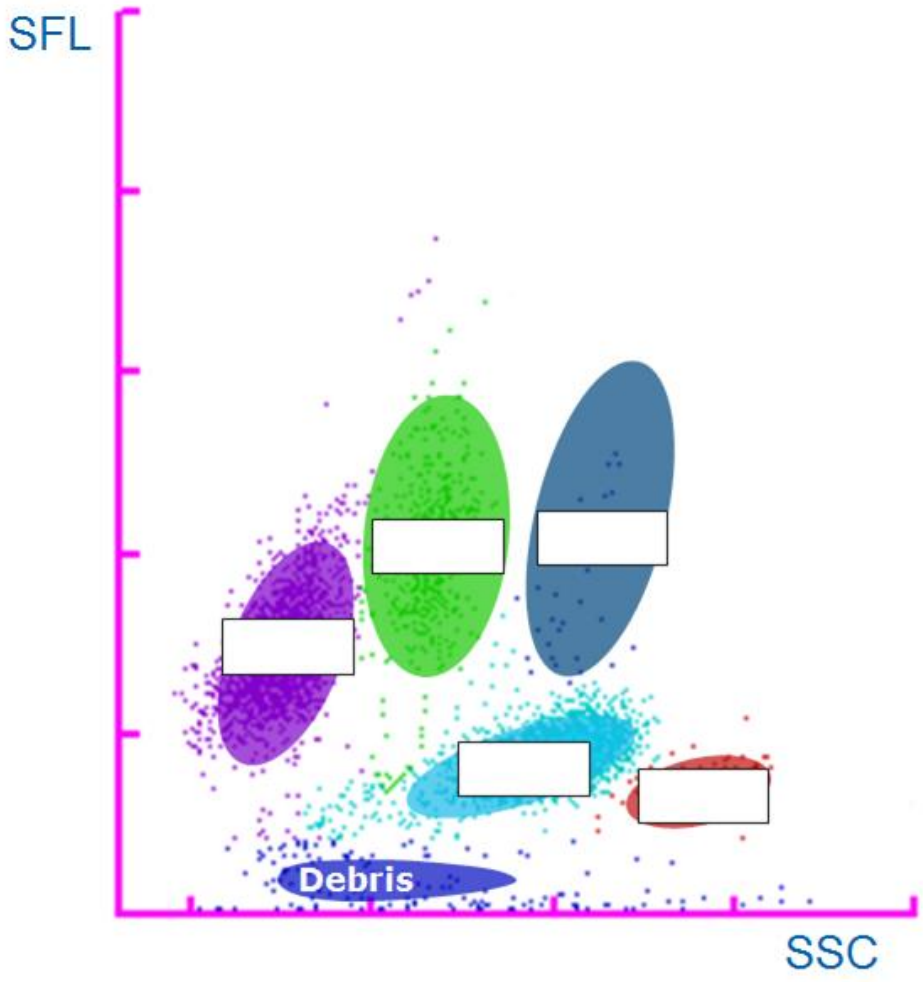
Using your knowledge of the XN-Series reagents and your understanding of fluorescence flow cytometry, please list the reagents used in this channel and label the scattergram below (including the axis).

Reagents used in this channel:

1. _____

2. _____

3. _____



RET Channel Scattergram - Activity 3:

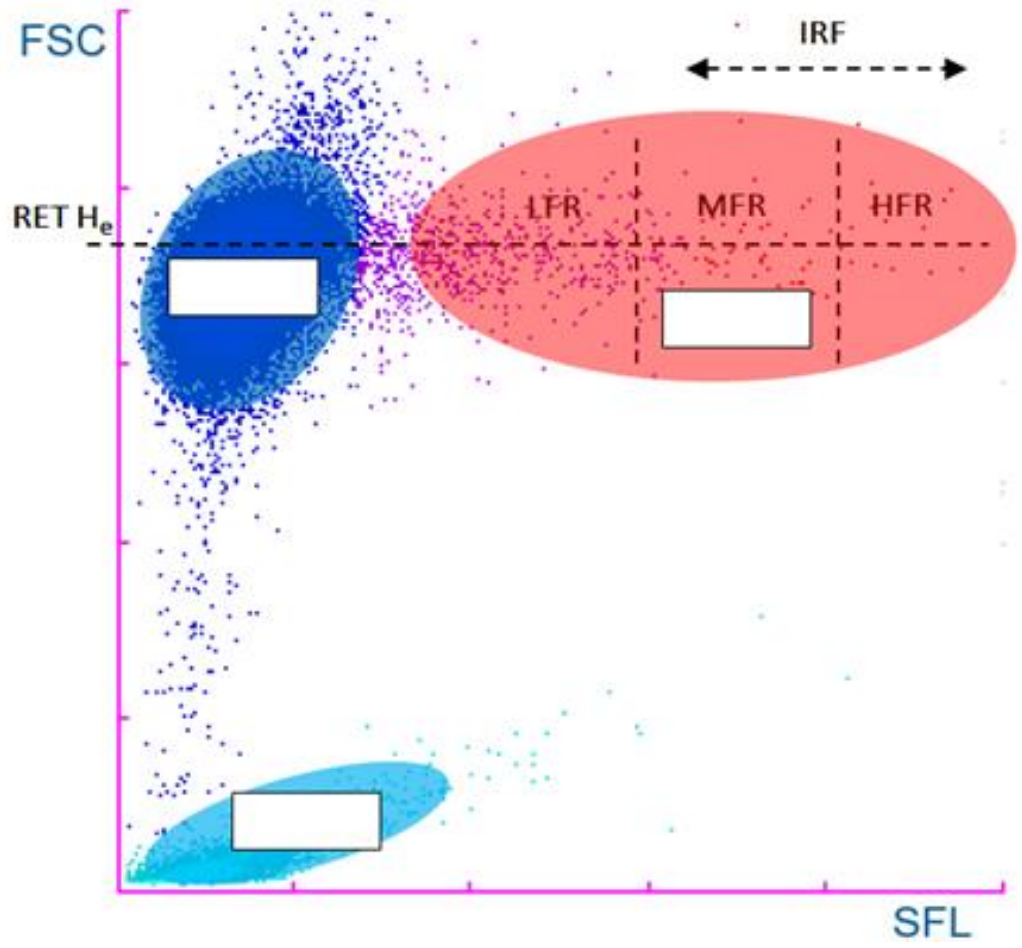
Using your knowledge of the XN-Series reagents and your understanding of fluorescence flow cytometry, please list the reagents used in this channel and label the scattergram below (including the axis).

Reagents used in this channel:

1. _____

2. _____

3. _____



PLT-F Channel Scattergram - Activity 4:

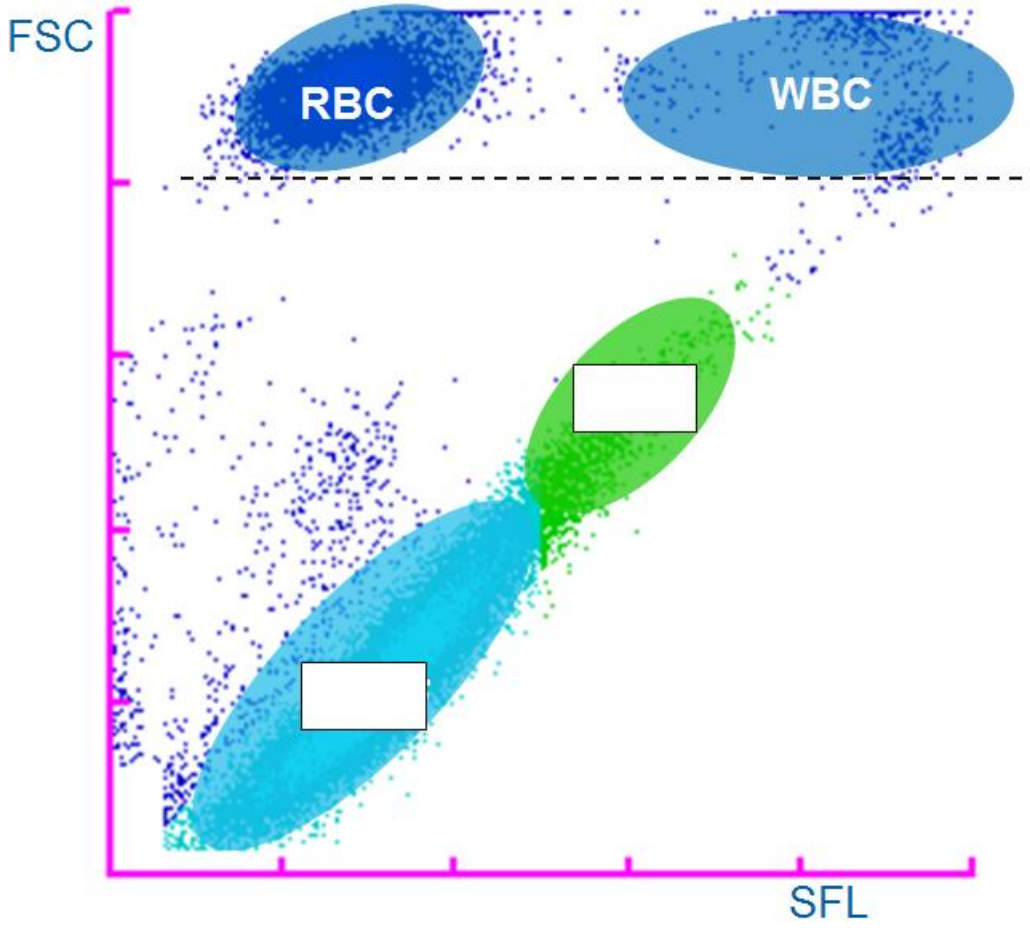
Using your knowledge of the XN-Series reagents and your understanding of fluorescence flow cytometry, please list the reagents used in this channel and label the scattergram below (including the axis).

Reagents used in this channel:

1. _____

2. _____

3. _____



WPC Channel Scattergram - Activity 5:

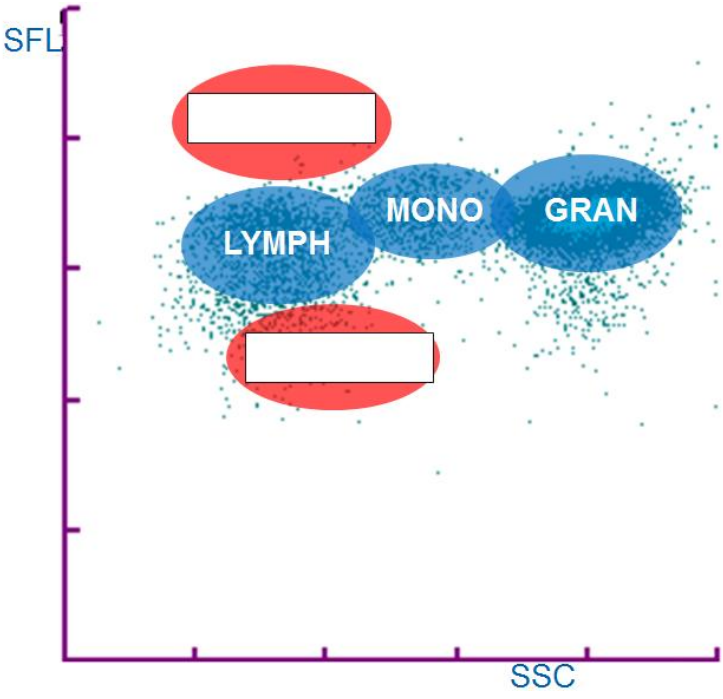
Using your knowledge of the XN-Series reagents and your understanding of fluorescence flow cytometry, please list the reagents used in this channel and label the scattergram below (including the axis).

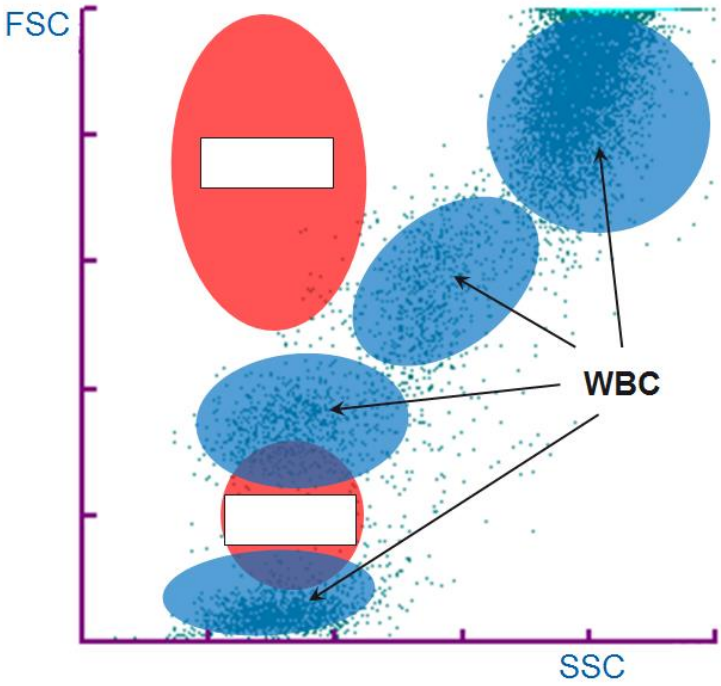
Reagents used in this channel:

1. _____

2. _____

3. _____





BF (Body Fluid) Channel Scattergram - Activity 6:

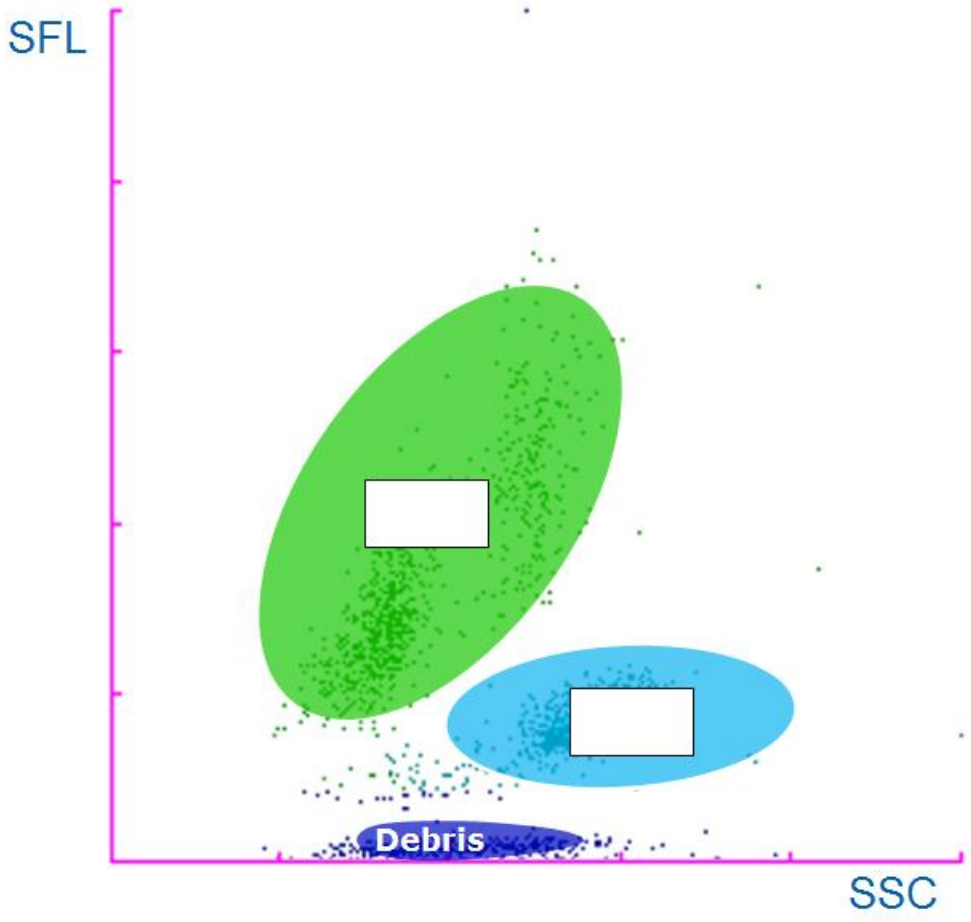
Using your knowledge of the XN-Series reagents and your understanding of fluorescence flow cytometry, please list the reagents used in this channel and label the scattergram below (including the axis).

Reagents used in this channel:

1. _____

2. _____

3. _____



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