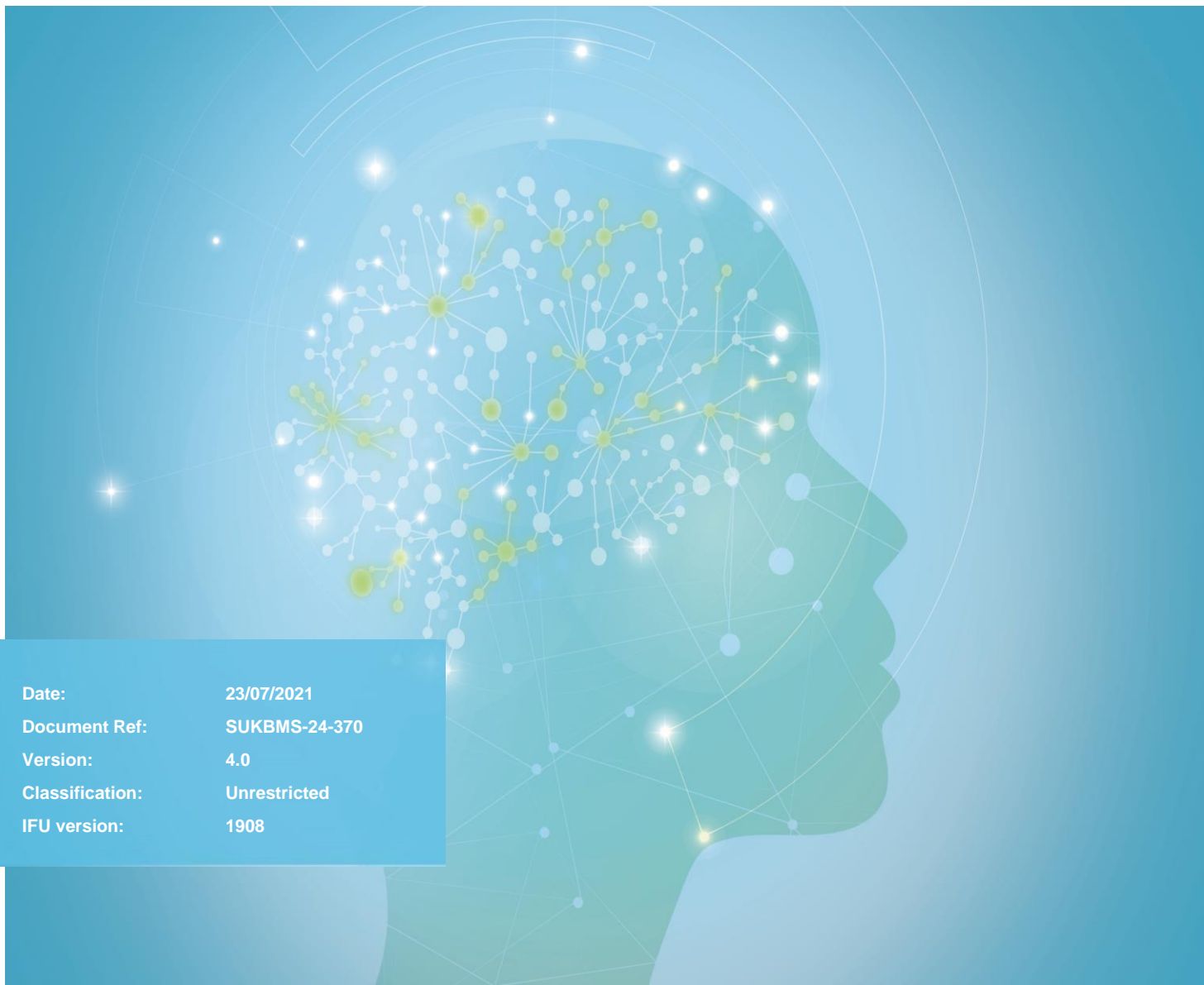


# Theory Training Workbook

## XN-V 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	J Hammersley	November 2019
All	Updated to reflect webinar content	N Bowen	May 2021
Contact Us	'Online' removed	N Bowen	July 2021

## Reference Documents

Document title	Version	Date
XN-1000V IFU	1812	December 2018
XN-2000V IFU	1812	December 2018

# Overview of XN-V Series

Facts and figures	
Analysers	XN-10V
Configurations	XN-1000V, XN-2000V
Analytical components	<p>Standard: 28 diagnostic parameters as standard</p> <div style="display: flex; justify-content: space-around;"> <div style="border: 1px solid black; padding: 2px; background-color: #4CAF50; color: white; text-align: center;">added value XN-CBC</div> <div style="border: 1px solid black; padding: 2px; background-color: #2196F3; color: white; text-align: center;">added value XN-DIFF</div> </div> <p>Optional: 16 optional diagnostic parameters</p> <div style="display: flex; justify-content: space-around;"> <div style="border: 1px solid black; padding: 2px; background-color: #F44336; color: white; text-align: center;">added value RET</div> <div style="border: 1px solid black; padding: 2px; background-color: #BBDEFB; color: black; text-align: center;">added value XN-BF</div> </div>
Technologies	Spectrophotometry, Sheath flow (DC) detection and Fluorescence flow cytometry
Modes of analysis * If available	Whole blood (WB) mode Low WBC (LW) mode Low Aspiration (LA) mode Pre-dilution (PD) mode Body fluid (BF) mode*
Aspiration methods	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 * If available	88µl (WB, LW, BF* modes) 70 µl (PD mode) 50 µl (LA mode)
Analysis range (whole blood mode)	WBC 0.00 to 440 x10 <sup>3</sup> /µL RBC 0.00 to 8.60 x10 <sup>6</sup> /µL Hb 0.0 to 260g/L HCT 0.0 to 75.0% PLT 0 to 5000 x10 <sup>3</sup> /µL NRBC# 0.00 to 20.00 x10 <sup>3</sup> /µl NRBC% 0.0 to 600.0/100WBC RET% 0.00 to 30.00% RET# 0.0000 to 0.7200 x10 <sup>6</sup> /µL
Species	<p>Standard: Mouse, Rat, Dog, NHP, Rabbit, Cat, Horse, Cattle, Pig, Mini Pig, Guinea Pig, Gerbil, Camel, Dolphin, Marmoset, Hamster, Ferret, Goat, Sheep</p> <p>Strain Species: Mouse 1-15, Rat 1-10, NHP 1-10, Rabbit, 1-5, Dog 1-5, Pig, 1-5</p> <p>Free Species 1-99</p>

# Reagents On-board the XN-V Series

There are a total of 8 different reagents that can be used on XN-V 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*
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	
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

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

# Analysis Principles and Parameter Production

The XN-V 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-V Series analysers. The XN-CBC channel is a standard channel on the XN-10V analyser.

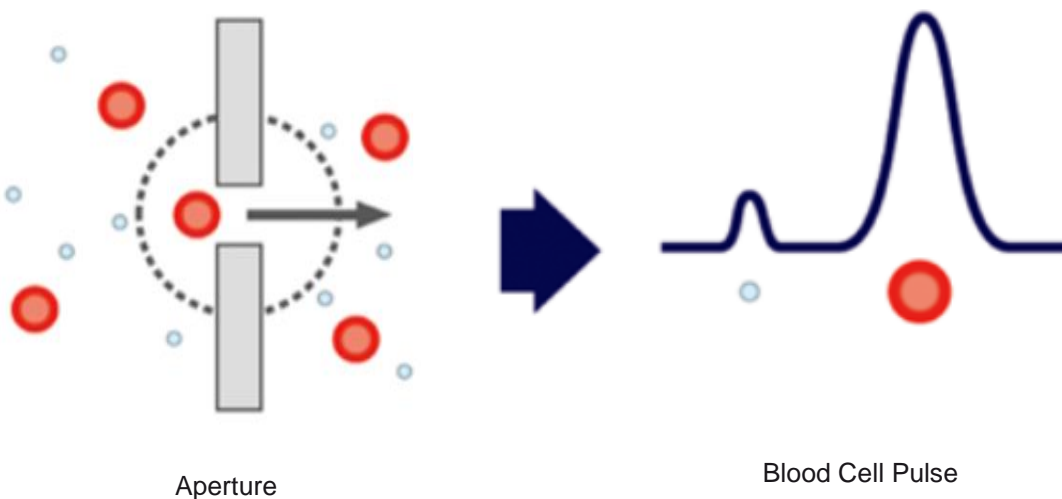
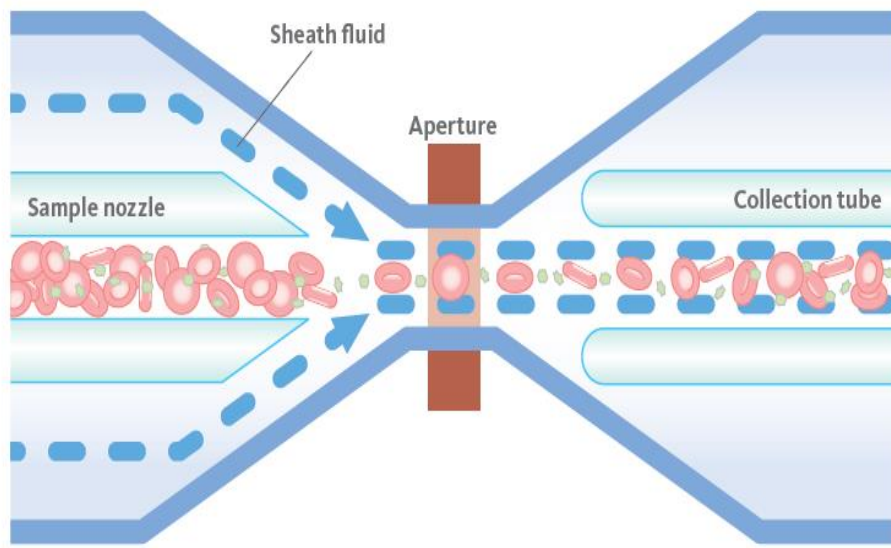
## CellPack DCL

CellPack DCL is a ready to use reagent for use with Sysmex analysers. CellPack DCL is used in the XN-CBC channel to produce RBC and platelet parameters via Sheath Flow (DC) Detection

CellPack DCL 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-V 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-V Series analysers use sheath flow (DC) detection for producing RBC and platelet parameters using CellPack DCL. 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).

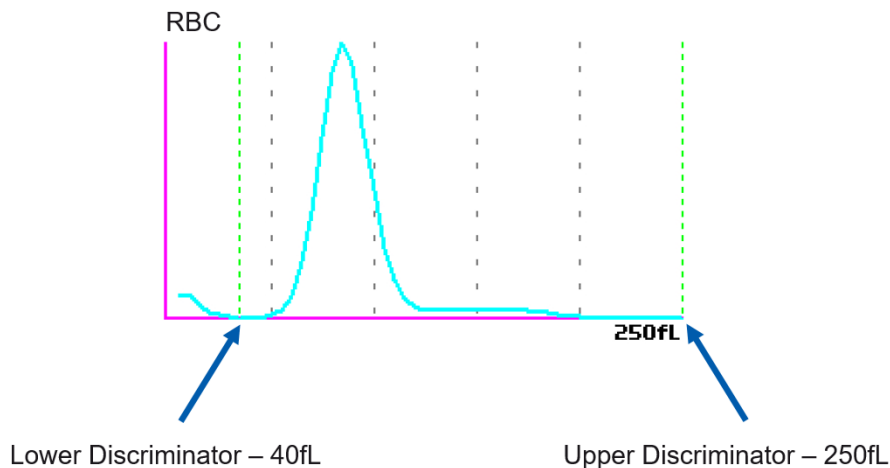




The RBC and PLT histograms are generated from this detection principle. The x-axis on the histogram relates to the size of the cell and the y-axis relates to the number of cells counted.

## RBC Histogram

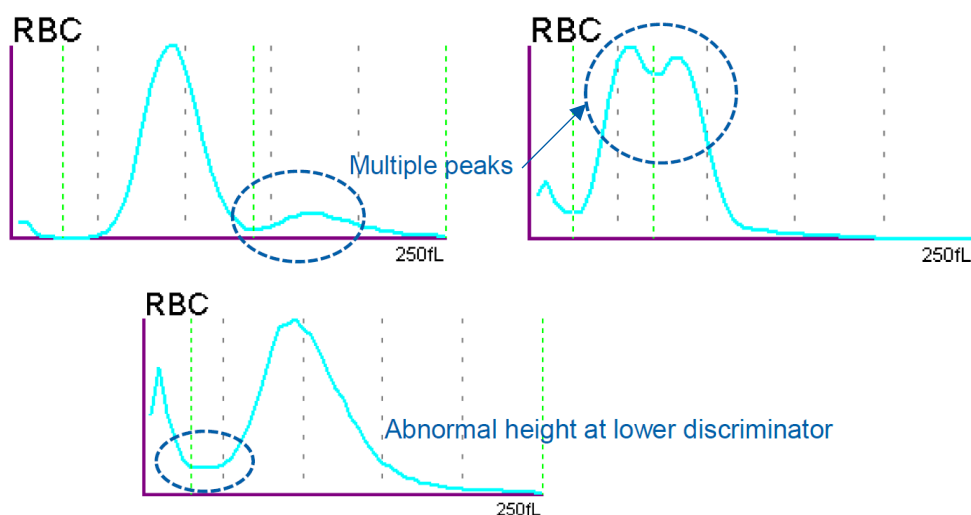
### Normal RBC Histogram



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.

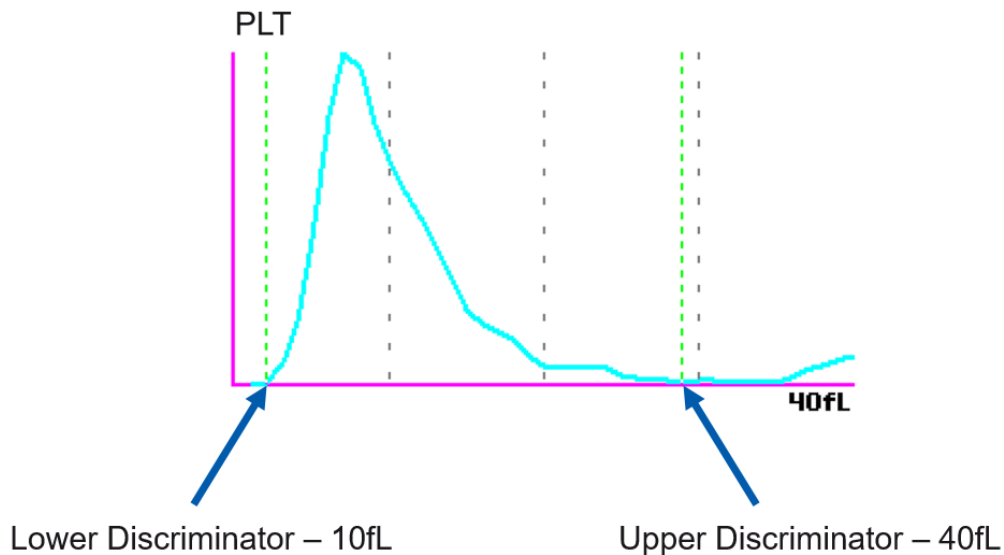
### Abnormal RBC Histogram

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



## PLT Histogram

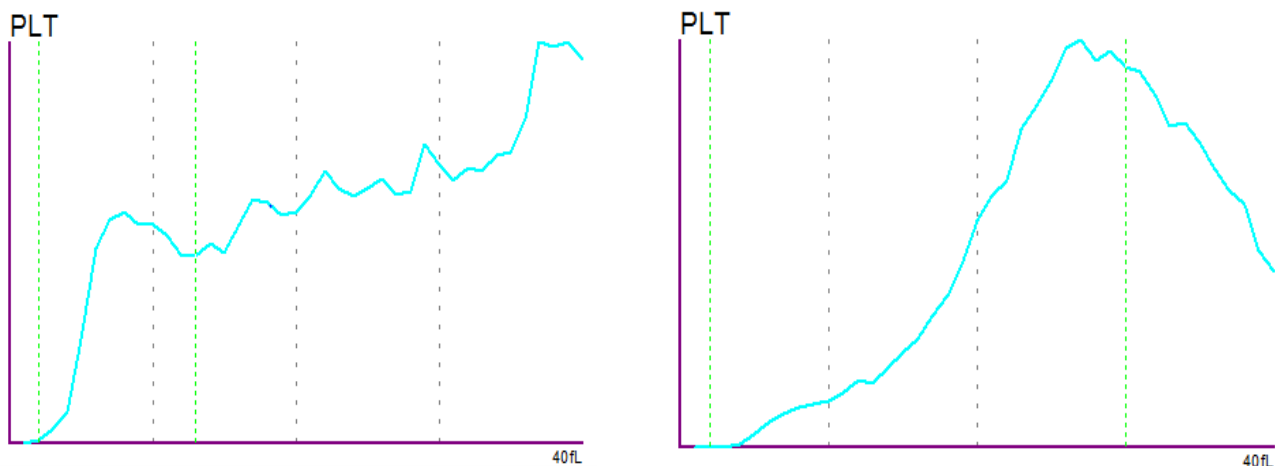
Normal PLT Histogram



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-V Series analysers. Any abnormal PLT histogram pattern that is generated will result in the PLT Abn Distribution flag being generated.





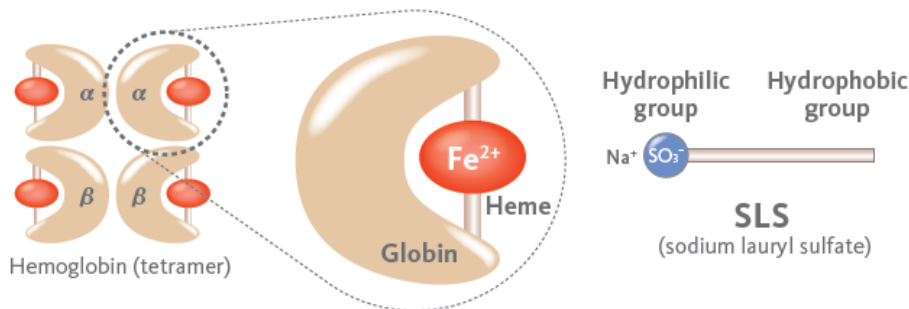
## Spectrophotometry

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

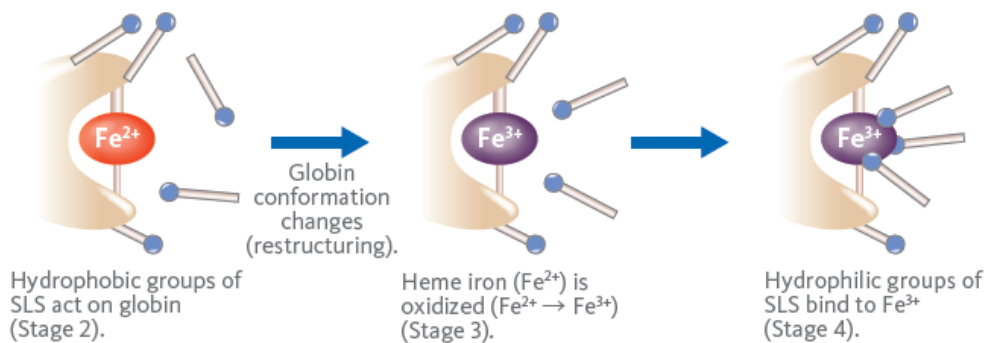
## Sulfolyser

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

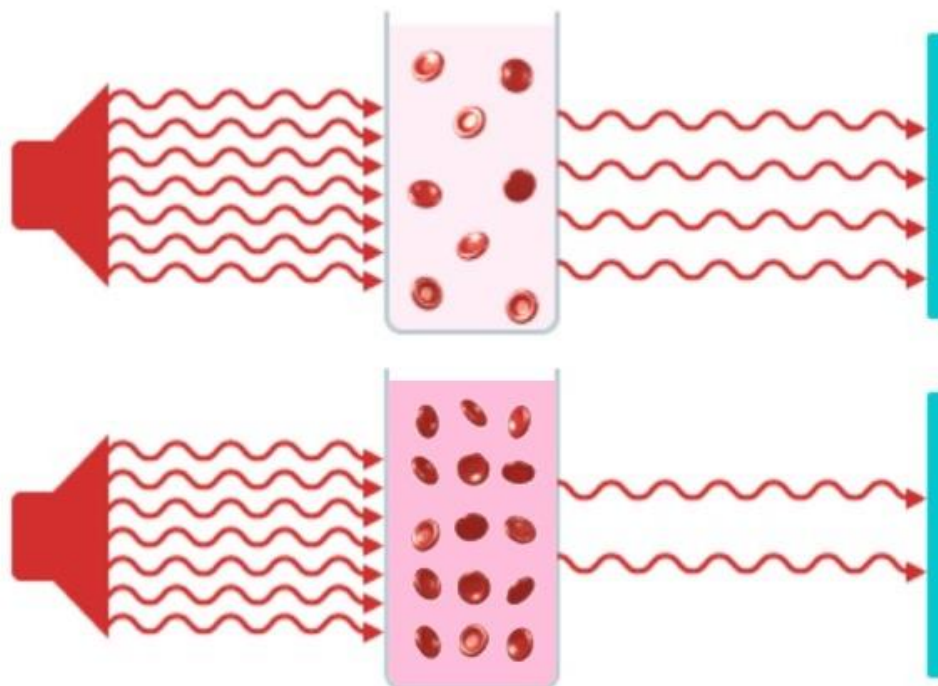
The key component of Sulfolyser is 'Sodium Lauryl Sulfate'. This molecule has hydrophobic / hydrophilic portions (see diagram), which is significant for its purpose.



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, the difference between the blank and the reaction complex is directly proportional 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:

$$\text{MCV (fL)} = [\text{HCT \%} \div \text{RBC (x10}^6/\mu\text{l)}] \times 10$$

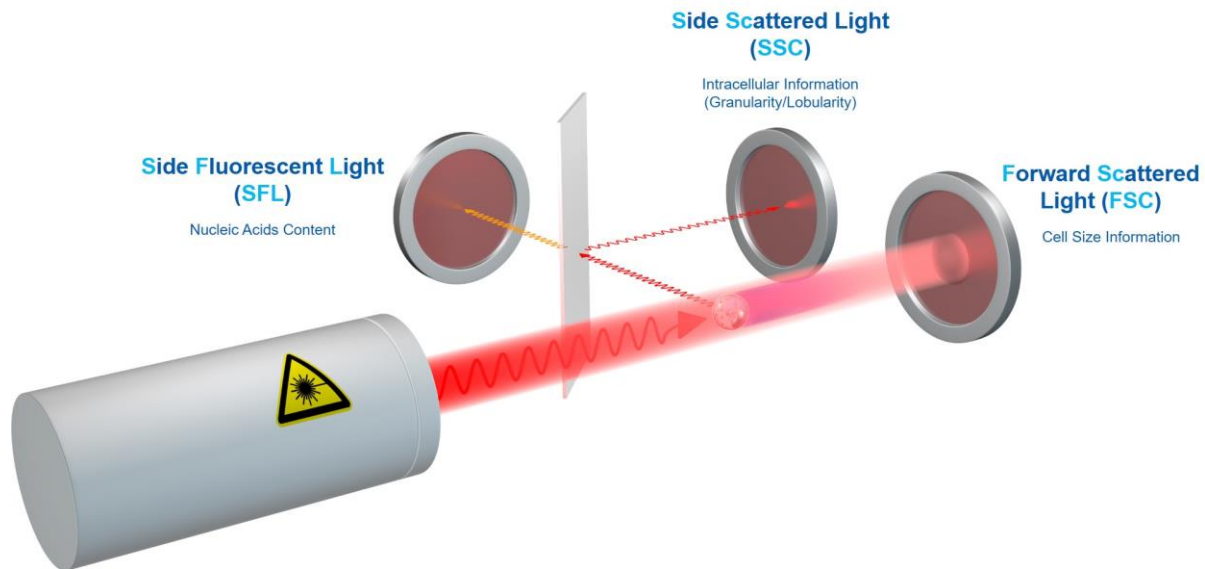
$$\text{MCH (pg)} = [\text{HGB (g/dL)} \div \text{RBC (x10}^6/\mu\text{l)}] \times 10$$

$$\text{MCHC (g/dL)} = [\text{HGB (g/dL)} \div \text{HCT (\%)}] \times 100$$



## Fluorescence Flow Cytometry

The XN-V 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-V Series channels make use of this technology:

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



## XN-CBC (WNR) Channel

The XN-CBC (WNR) is a standard channel on the XN-10V analyser. Fluorescence flow cytometry within the XN-CBC (WNR) Channel is used for enumeration of WBCs (non-basophil), basophils and NRBCs, using CellPack DCL (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.

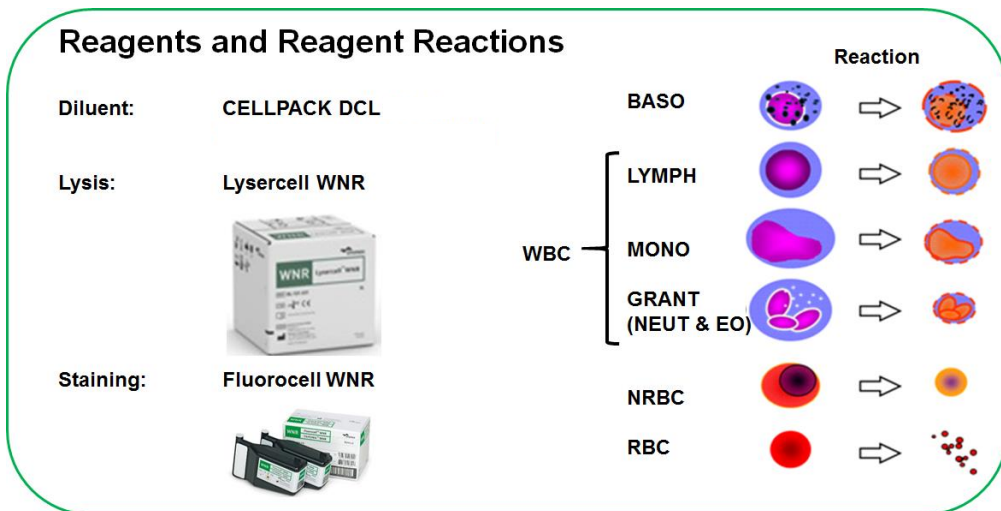
It shrinks all of the WBCs except the basophils which remain in a near native state.

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



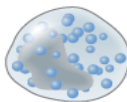
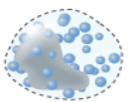
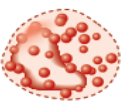




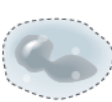

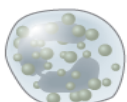
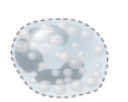







#### Fluorocell WNR

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)

	Penetration of the cell membrane Hemolysis		Fluorescence		Side fluorescent light (SFL)	Forward scattered light (FSC)	
<b>Basophils</b>		→		→		Strong	Strong
<b>Lymphocytes</b>		→		→		Medium	Medium
<b>Monocytes</b>		→		→			
<b>Granulocytes (neutrophils, eosinophils, etc.)</b>		→		→			
<b>Nucleated red blood cells</b>		→		→		Weak	Medium
<b>Red blood cells</b>		→		→		Very weak	Very weak

※ This is a conceptual drawing.

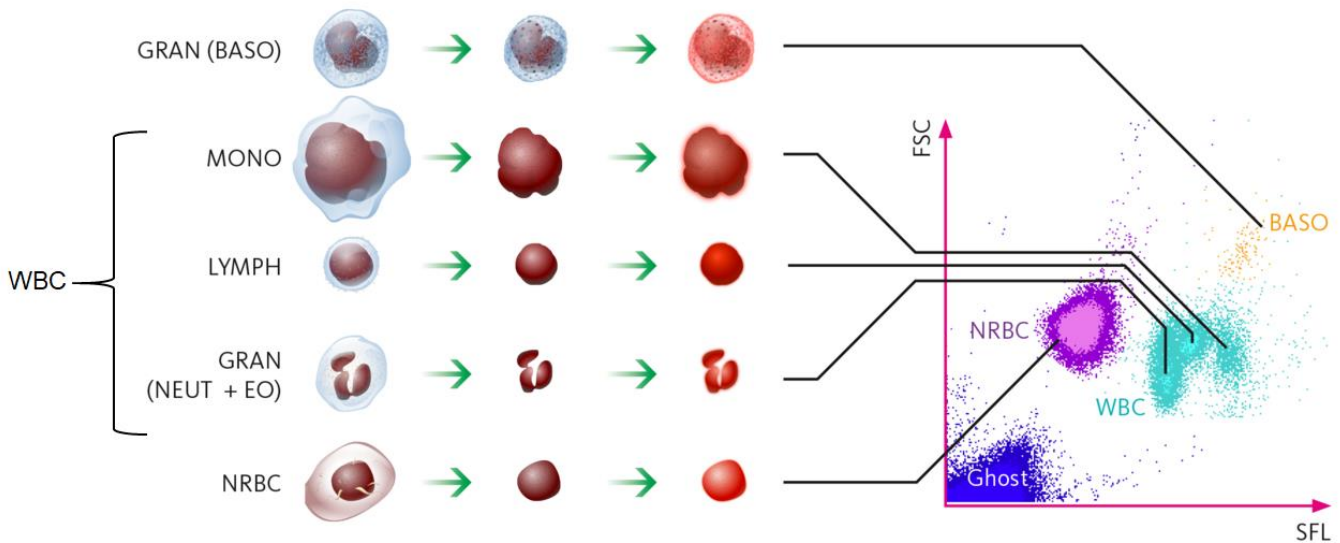
Populations:

**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-V 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-V 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.





## XN-DIFF (WDF) Channel

The XN-DIFF (WDF) is a standard channel on the XN-10V analyser. Fluorescence flow cytometry within the XN-DIFF (WDF) Channel is used to differentiate neutrophils, lymphocytes, monocytes and eosinophils, using CellPack DCL (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



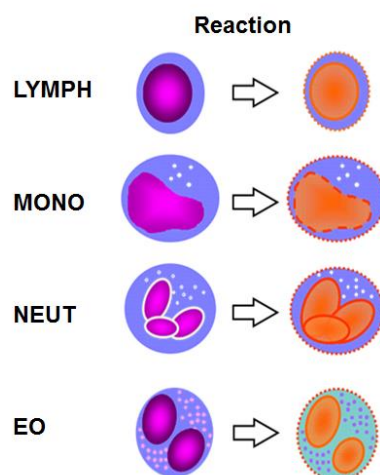
### Reagents and Reagent Reactions

Diluent: **CELLPACK DCL**

Lysis: **Lysercell WDF**







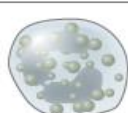

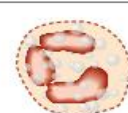
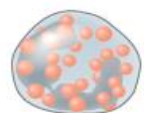
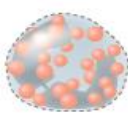



Staining: **Fluorocell WDF**



## 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).

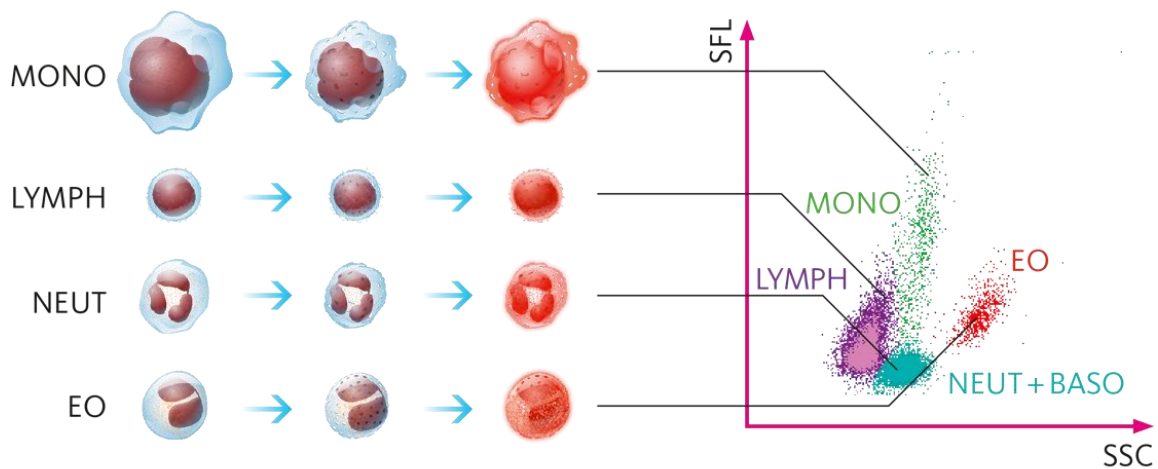
		Hemolysis		Staining		Side fluorescent light (SFL)	Side scattered light (SSC)
Lymphocytes		→		→		Medium	Weak
Monocytes		→		→		Medium	Weak
Neutrophils		→		→		Weak	Medium
Eosinophils		→		→		Weak	Strong

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.



The WDF Channel is used to report the following parameters:

- Lymphocyte # and %
- Monocyte # and %
- Neutrophil # and %
- Eosinophil # and %

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-V 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.

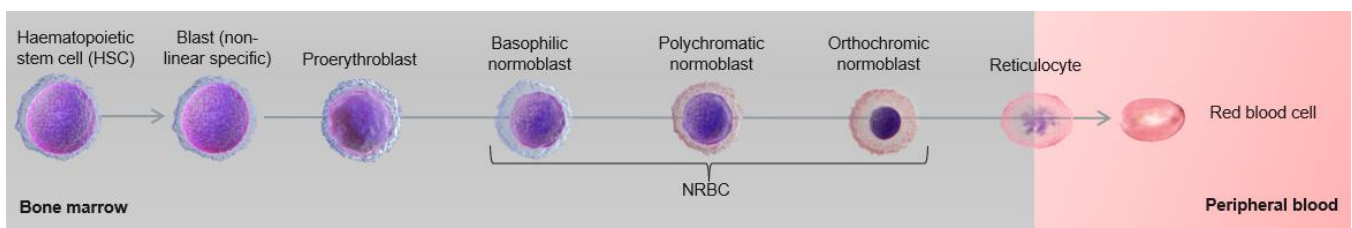
The white cell parameters produced in low white cell mode (LW) are generated from the WDF channel, using the same reagents and include:

- 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 %

added value  
**RET**

## RET Channel

The XN-RET channel is an optional channel for the XN-10V 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 (as an isotonic diluent), CellPack DFL and Fluorocell RET.

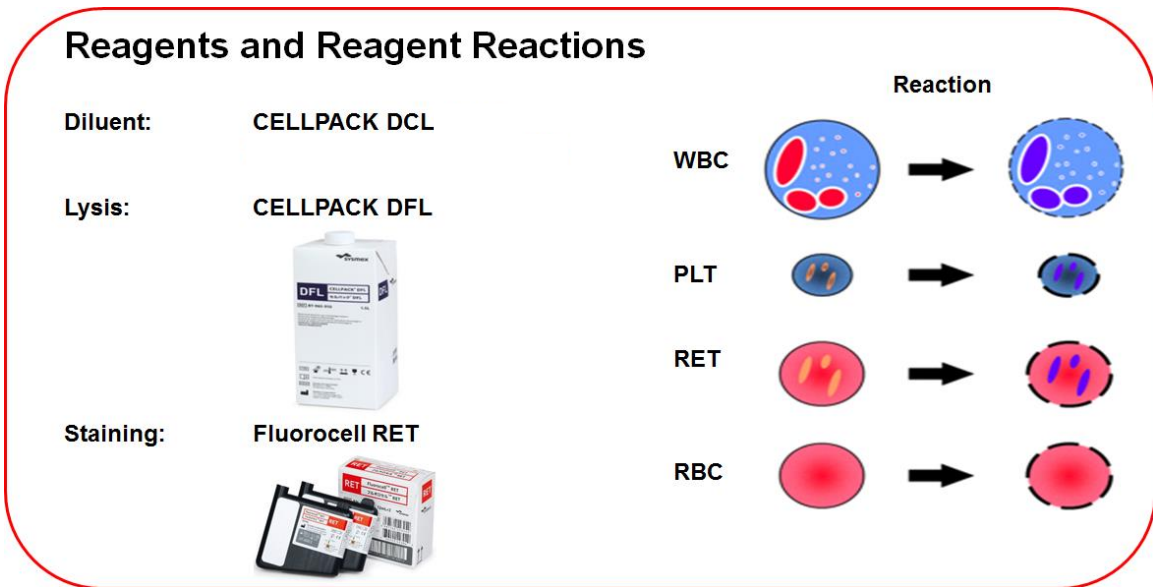
### Reagents

#### 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.

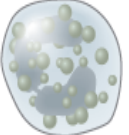





#### Fluorocell RET

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.

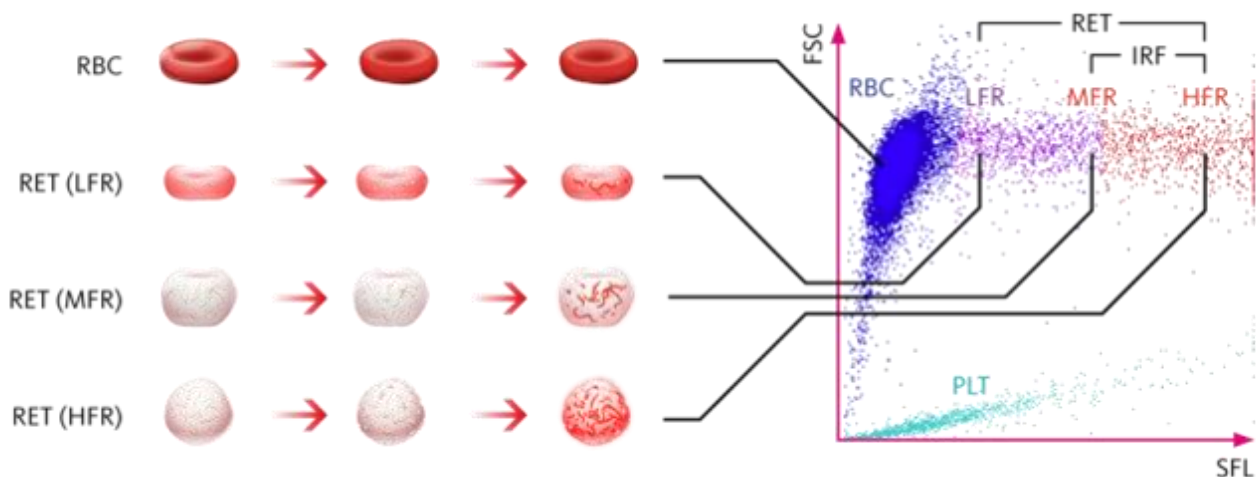


Populations:

**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<sub>e</sub>: RET-H<sub>e</sub> 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-V 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.

added value  
XN-BF

## BF (Body Fluid) Mode

The XN-BF mode is an optional application for the XN-10V analyser. 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 to perform the RBC count using sheath flow (DC) detection in the XN-CBC channel and CellPack DCL (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

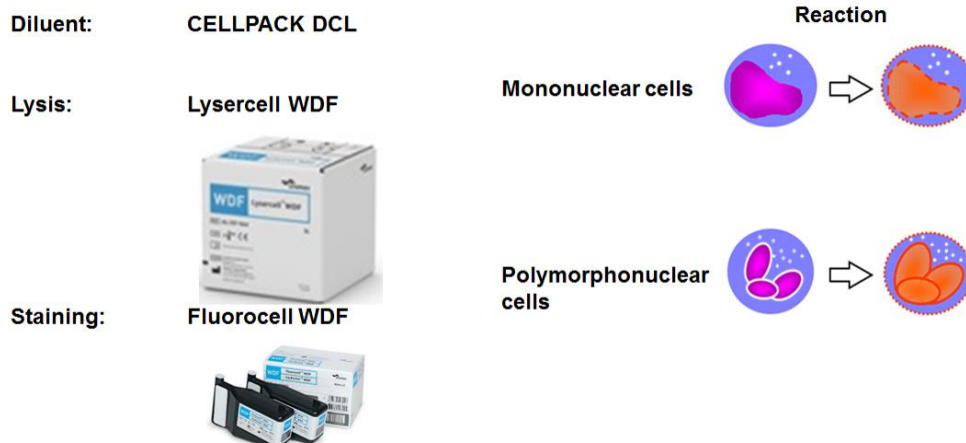
The Lysercell WDF reagent haemolyses red blood cells and the membranes of WBCs are made permeable to allow the Fluorocell WDF to enter the cells.

#### 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



### Reagents and Reagent Reactions



## 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).

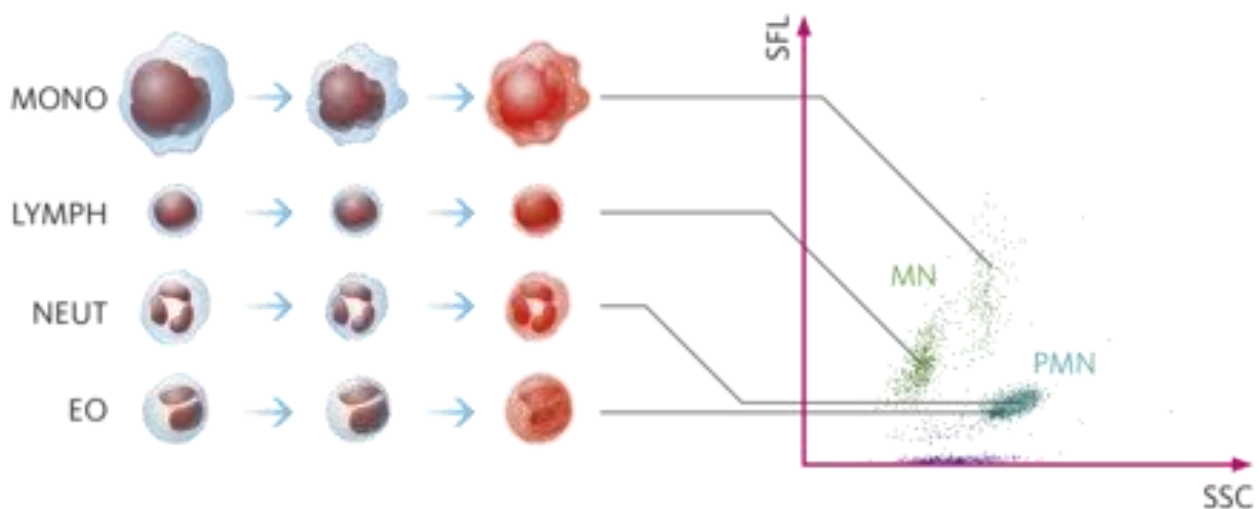
Populations:

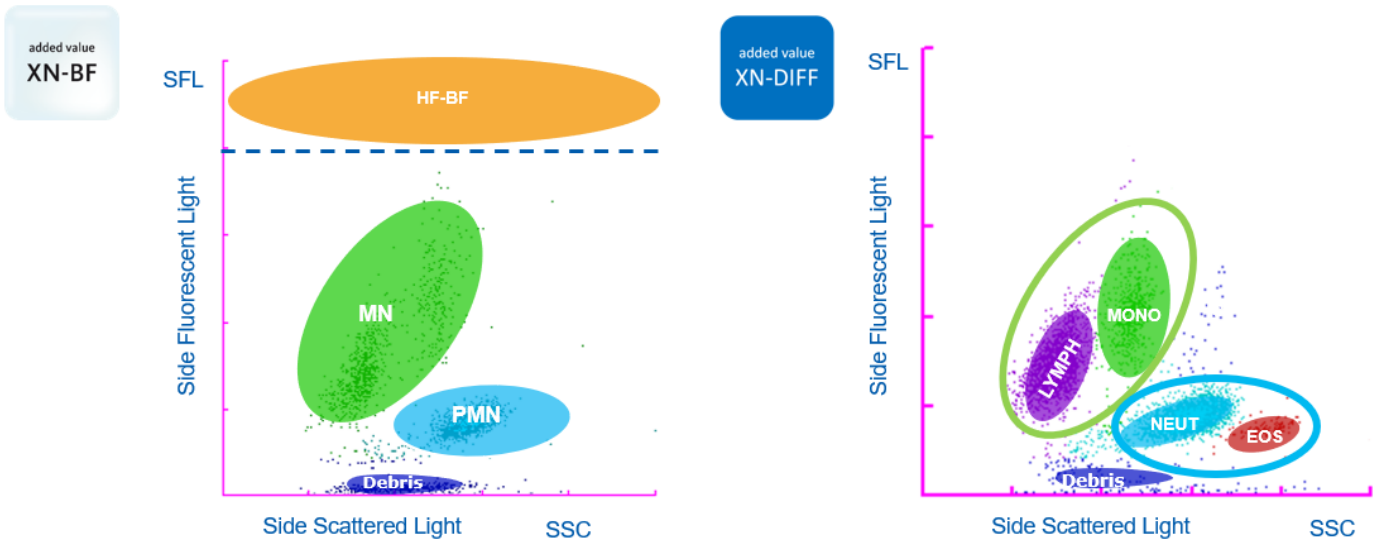
**Mononuclear Cells (MN):** Mononuclear cells (lymphocytes and monocytes) display medium to weak SFL and weak SSC.

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

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.

# XN-V Series Flagging

The XN-V Series software is designed to aid in the separation of specimens into POSITIVE and NEGATIVE categories according to pre-set criteria. The system bases its judgments on comprehensive surveys of numerical data, particle size distributions, scattergrams, and provides easy-to-understand flags/messages indicating the instruments findings. These flags and messages are referred to as Interpretive Program (IP) Messages.

A specimen is judged NEGATIVE when there are no predefined abnormalities present in the specimen. The results are generally reported without review.

The XN-V Series analysers will generate a POSITIVE when an Interpretive Program (IP) Message is present. An established review process by lab personnel should be initiated. These results should be reviewed carefully and may require further examination in accordance with the protocol of your laboratory. POSITIVE flags can be separated into two types; Abnormal and Suspected.

Abnormal	Suspected
Indicates that the sample is clearly abnormal	Indicates a possibility that the sample is abnormal
Customisable	Non-customisable
Parameter marks: @, *, !, +/-, +++, ---- Interpretive messages: neutrophilia, lymphopenia, monocytosis, dimorphic	Q-Flags: Platelet clumps?

## Abnormal Flags

Abnormal flags indicate that a sample is clearly abnormal and are customisable for individual species settings and are largely related to numbers; such as neutrophilia, microcytosis or thrombocytopenia.

### WBC Abnormal Flags

Message	Judgement method/equation
WBC Abn Scattergram	Based on clustering in the WNR and WDF scattergrams. For body fluid analysis, based on clustering in the WDF scattergram
Neutropenia	Judged from conditions set in species settings
Neutrophilia	
Lymphopenia	
Lymphocytosis	
Monocytosis	
Eosinophilia	
Basophilia	
Leukocytopenia	
Leukocytosis	
NRBC Present	

RBC Abnormal Flags

Message	Judgement method/equation
RBC Abn Distribution	Judged from RBC distribution
Dimorphic Population	Gap between the high and low points and shape of distribution peak
RET Abn Scattergram	Clustering in the RET scattergram
Reticulocytosis	Judged from conditions set in species settings
Anisocytosis	
Microcytosis	
Macrocytosis	
Hypochromia	
Anaemia	
Erythrocytosis	

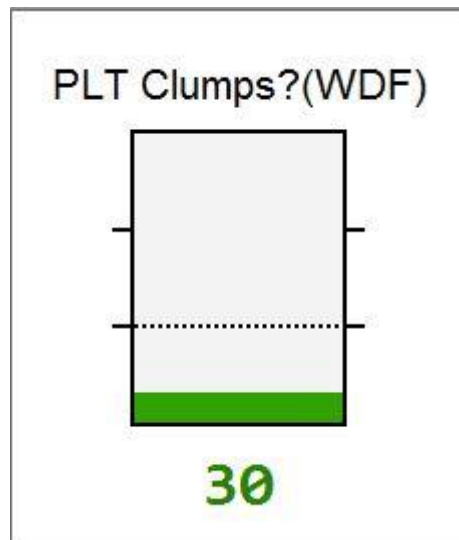
PLT Abnormal Flags

Message	Judgement method/equation
PLT Abn Distribution	Judged from PLT distribution
PLT Abn Scattergram	PLT clustering in the PLT scattergram
PLT Clumps?	
Thrombocytopenia	Judged from conditions set in species settings
Thrombocytosis	

## Suspected Flags

Suspected flags indicated a possibility that the sample is abnormal and are generated when the Q-flag reaches the trigger level.

The only suspected flag generated by the XN-V Series is PLT Clumps?, which indicates the possibility of platelet clumps and is judge by assessing activity in the PLT clump flagging region on the WDF scattergram. The number generated is arbitrary and generated from light signals in the flow cell. The flag is triggered when this figure reaches 100.





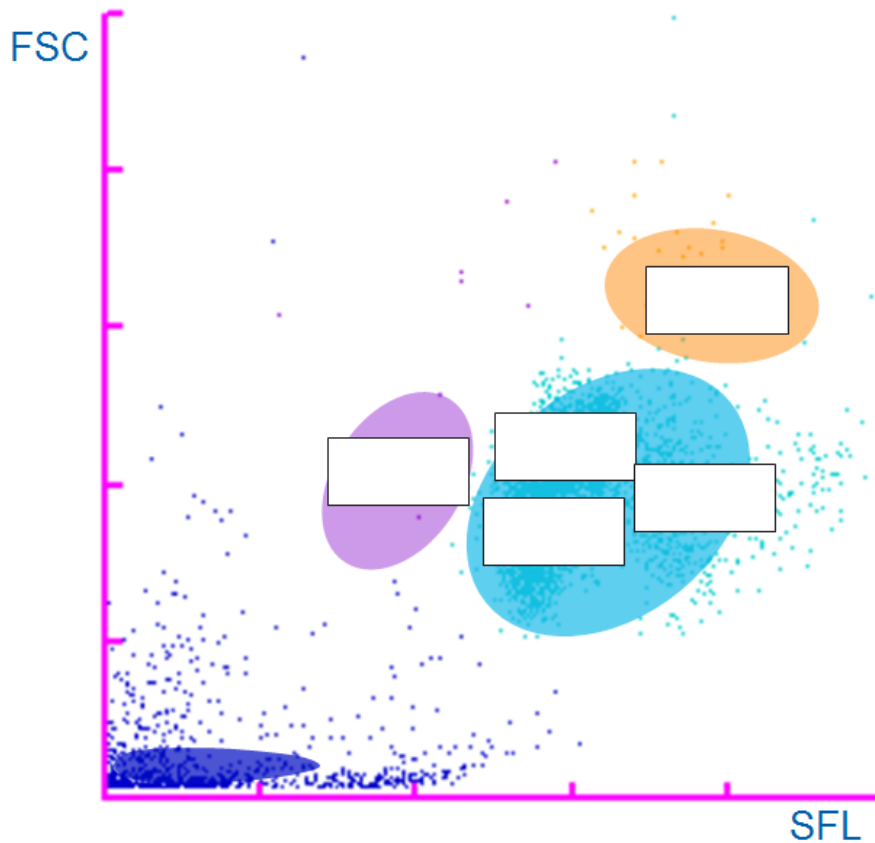
# Activities

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

Using your knowledge of the XN-V 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. \_\_\_\_\_

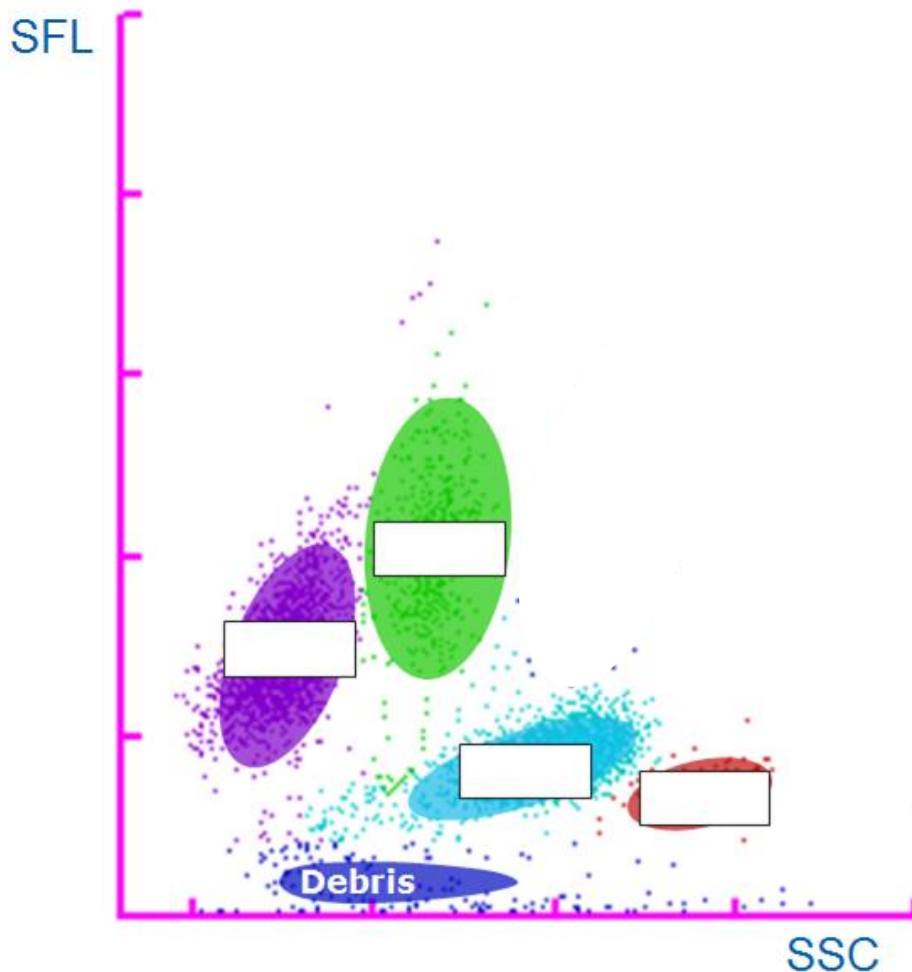


XN-DIFF (WDF) Channel Scattergram - Activity 2:

Using your knowledge of the XN-V 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. \_\_\_\_\_

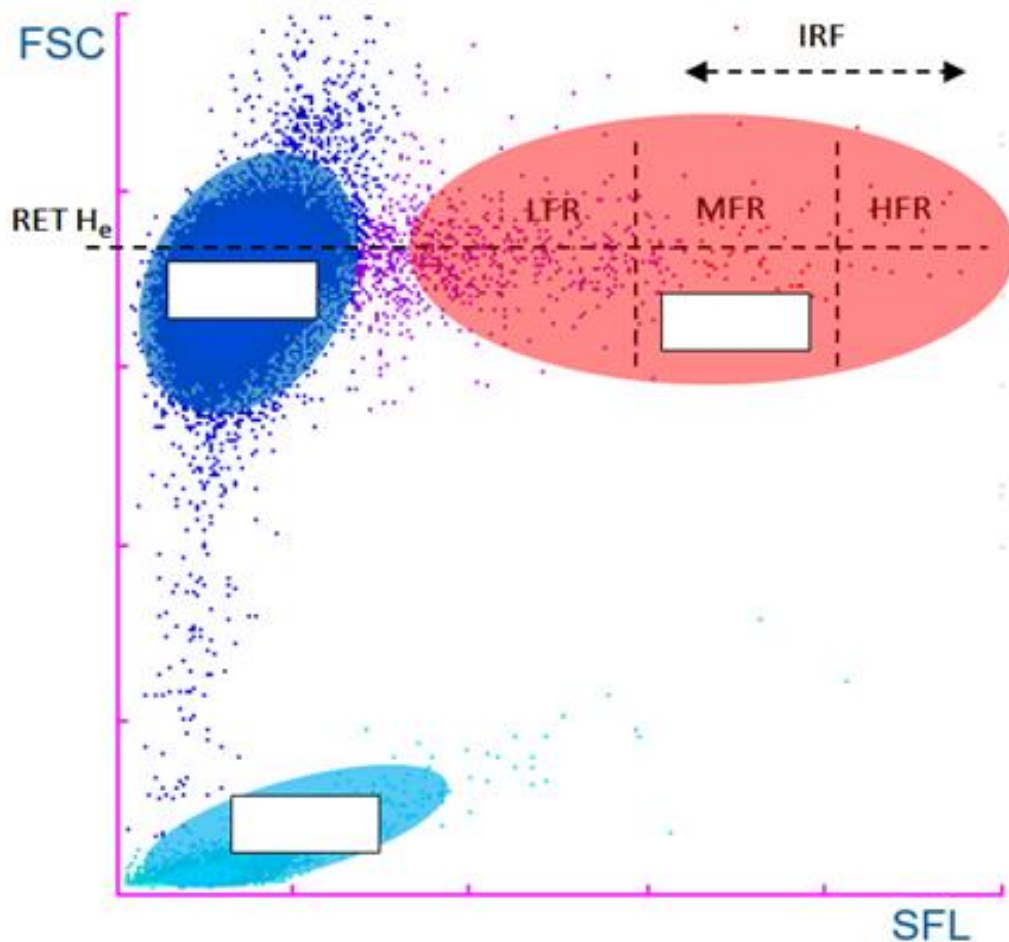


RET Channel Scattergram - Activity 3:

Using your knowledge of the XN-V 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. \_\_\_\_\_

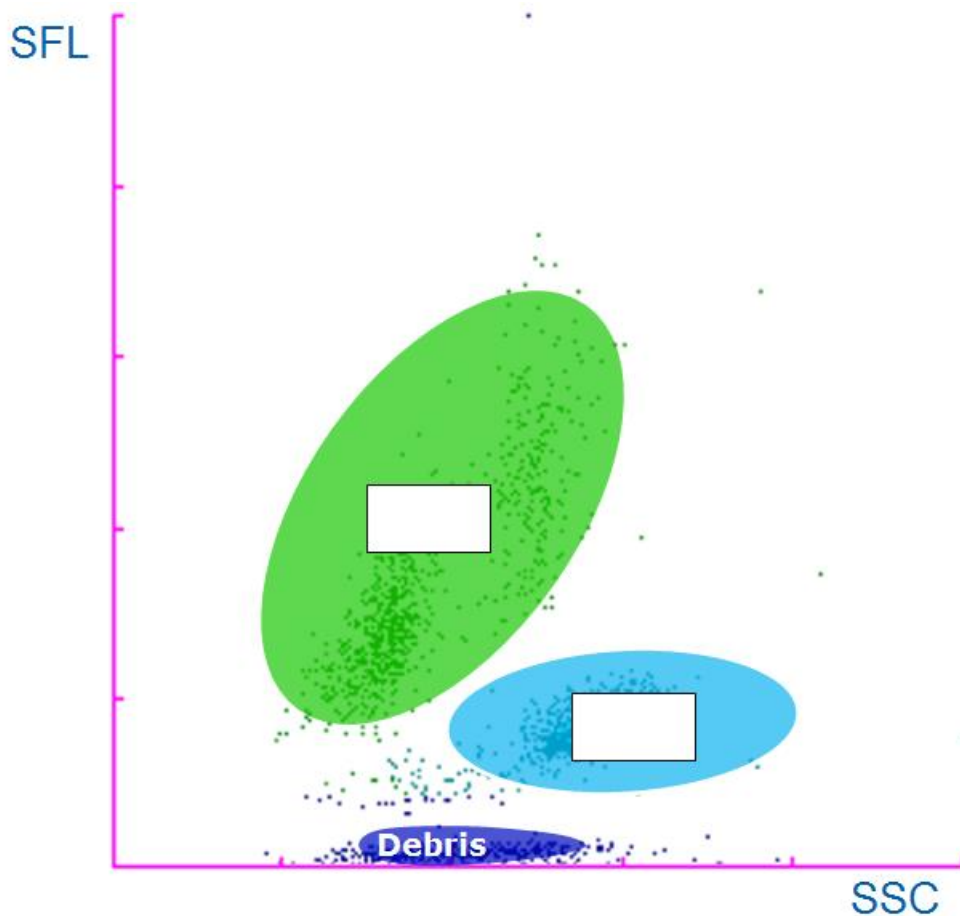


BF (Body Fluid) Channel Scattergram - Activity 4:

Using your knowledge of the XN-V 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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