



Cell functionality

Going beyond the visible: Reliable characterisation of WBC functionality

Inside the cell membrane

Investigations into the cellular membrane composition date back to the 1880s when Overton discovered the presence of a lipid boundary layer in 1889. Further discoveries followed, creating the concrete image of the membrane components we know of today.

Cell membranes are composed of a lipid bilayer made of two opposing leaflets. Within these leaflets, numerous lipid species interact with proteins regulating the dynamic membrane bioactivity. More specifically, lipid rafts are submicroscopic specialised regions of the cell membrane made up of unique components. They are rich in cholesterol and saturated fatty acids (Fig. 1). These two constituents contribute greatly to the rigidity, tight compact structure and high dynamism of lipid rafts. In fact, lipid rafts were initially referred to as 'detergent-resistant membrane fractions' because of their tightly packaged detergent-resilient constituents. Interestingly, these assemblies are very dynamic and can vary their clustering depending on cell activation and maturation stage as well as in malignancy. Lipid rafts were reported to be present at elevated levels in cells with active extracellular communication, such as active T cells [1], and in cancer cells [2].

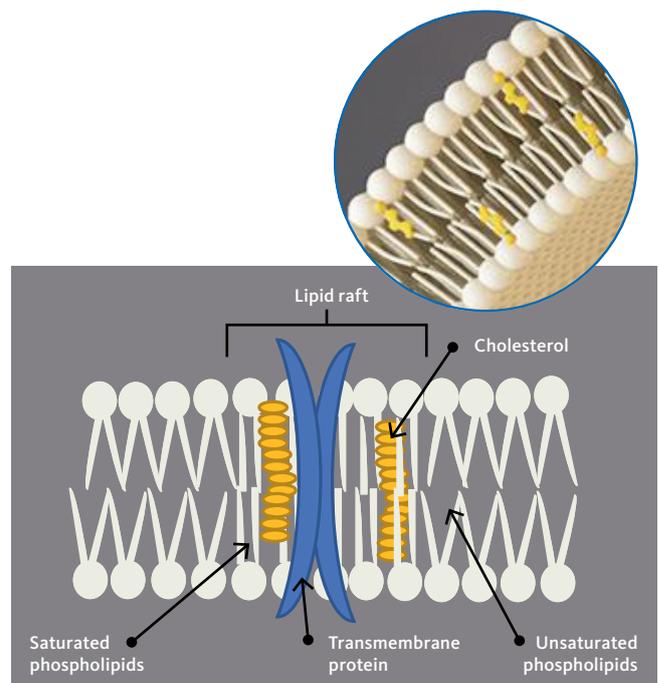


Fig. 1 Cell membrane composition showing the contents of lipid rafts

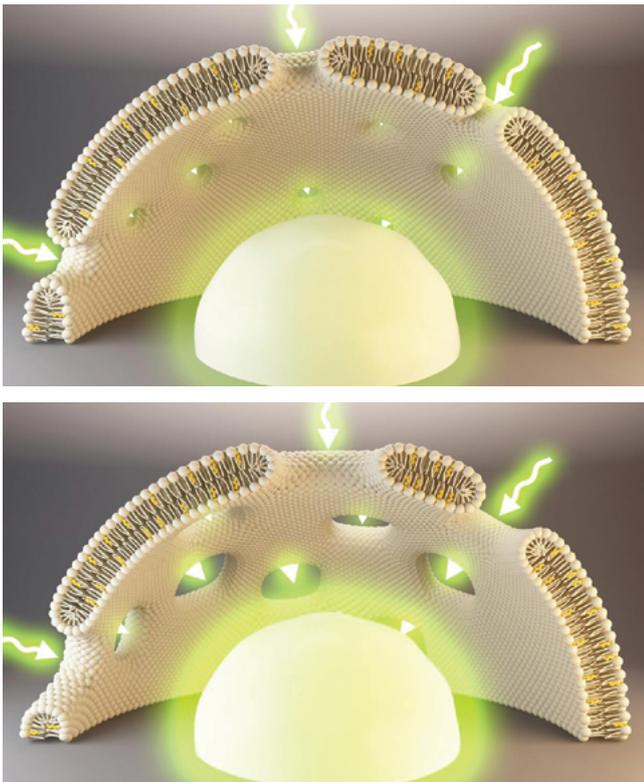


Fig. 2 Cell membrane after being treated with Sysmex lysing and fluorescence reagents. Depending on the lipid composition, the membrane is perforated differently, allowing moderate (top) or high (bottom) fluorescence labelling.

Furthermore, studies showed that cholesterol plays a major role in the resistance of the cell membrane to non-ionic reagents such as Triton X-100 [3], whereby the quantity of cholesterol implies the degree of resistance.

This observation has been put to use in Sysmex reagents containing non-ionic detergents. The desired effect is that these reagents perforate a cell's membrane in a specific way, depending on its composition and activity status.

The discriminating power of Sysmex XN-Series' and XR-Series' fluorescence channels stems from their ability to provide information about the cell membrane composition and the internal cellular structure. Depending on the lysing reagent being used, the cell membrane is perforated differently with its lipid rafts remaining almost intact. Following the perforation, the fluorescence reagent can enter the cell and will specifically label certain components, thereby generating unique, channel-specific information (Fig. 2).

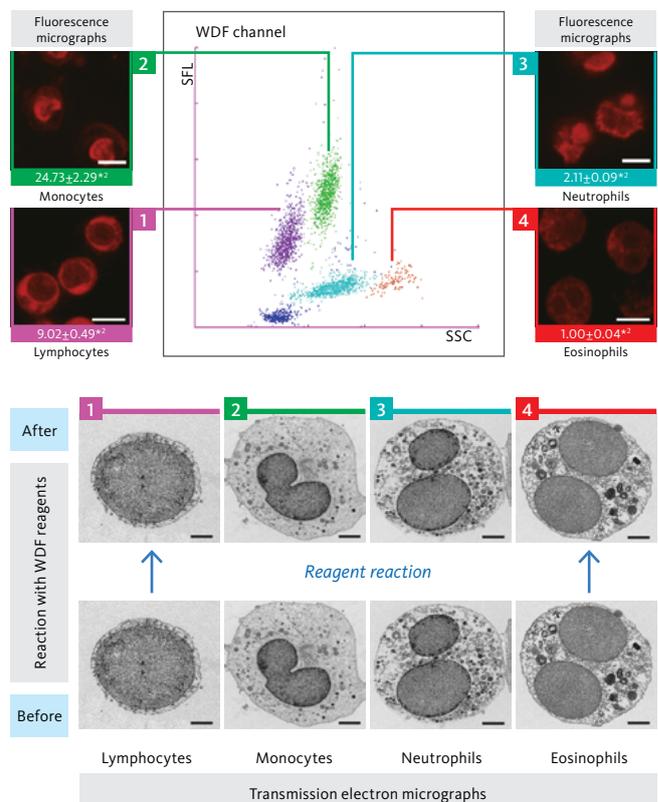
The fluorescence of the cell depends on

1. the degree of permeabilisation,
2. its DNA content, and/or
3. its RNA content.

The operating principle of the WDF channel

The white blood cell differential (WDF) channel utilises fluorescence markers that can separate different WBC subtypes according to their cell membrane composition and cytoplasmic content. As described above, the lysing reagent first perforates the cell membrane while leaving the cells largely intact. Then, the intracellular RNA is labelled with the fluorescence marker.

In a study by Kawauchi *et al.* [4], the morphology of WBC was examined before and after treatment with the WDF channel reagents, in particular with a view to the effect of these reagents on the different WBC subtypes and the resulting fluorescence intensity. Furthermore, the internal structure of these cells was investigated using transmission electron microscopy (Fig. 3). Compared with other WBC, lymphocytes have the least complex internal structure. This explains why they have a low side scatter (SSC) signal and are clearly separated from the other normal WBC subtypes. Interestingly, a reactive lymphocyte can also be differentiated from a normal one due to its increased cytoplasmic activity (RNA content). Monocytes with their bigger size and higher RNA content show a very high fluorescence intensity.



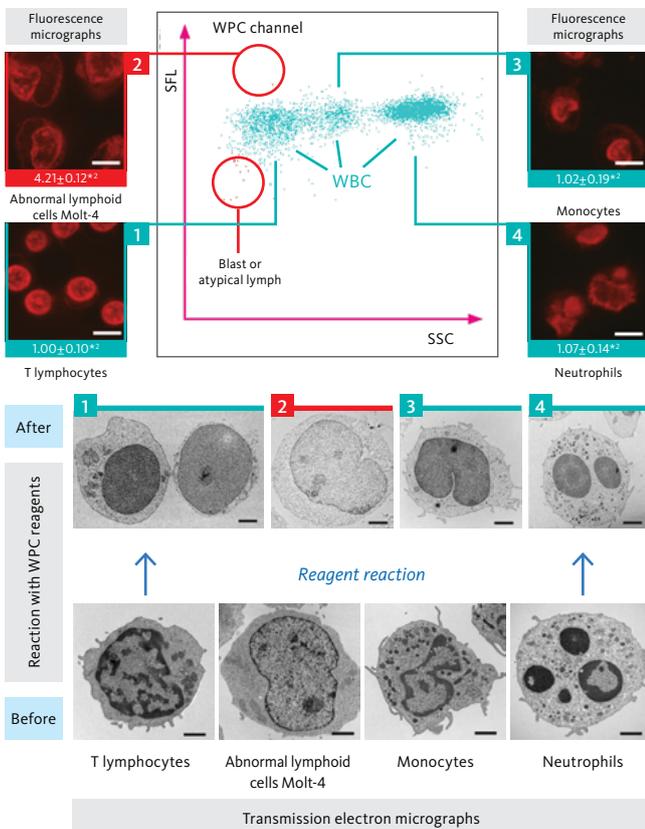
^{*2} Image analysis was performed and the fluorescence intensity (mean ± SD) of each white blood cell subtype was expressed, taking the mean fluorescence intensity of eosinophils as 1.0.

Fig. 3 Subtypes of WBC differentiated by the WDF channel. Populations of lymphocytes, monocytes, neutrophils and eosinophils are separated after the labelling of their RNA and depending on their intracellular complexity and size. Adapted from Kawauchi *et al.* [4].

... and of the WPC channel

The white precursor and pathological cell (WPC) channel also uses its reagents to distinguish between the different subtypes of WBC. The lysing reagent of the WPC channel, however, has a stronger impact on membrane lipids than the one used in the WDF channel. This is due to a different surfactant and the longer incubation period. Consequently, this treatment results in a higher level of cell membrane permeability. Furthermore, the fluorescence reagent of the WPC channel has a higher polymethine concentration than that of the WDF channel, allowing the labelling of DNA inside the nucleus instead of just cytoplasmic RNA.

The higher the degree of membrane perforation by the WPC lysing reagent, the more cellular content leaks through the pores. This results in a smaller cell size, more fluorescence marker entering the cell and binding to the DNA, and finally higher fluorescence intensity. For instance, non-reactive lymphocytes have the least complex structure and are smaller than the other WBC subtypes; therefore, they can be easily separated from the other mature normal WBC in the scattergram. Immature cells can be separated from mature ones due to their membrane lipid composition. The low lipid composition of such cells (stem cells, blasts, etc.) makes them more resistant to permeabilisation, finally resulting in lower fluorescence signals.



*2 Image analysis was performed and the fluorescence intensity (mean±SD) of each white blood cell subtype was expressed, taking the mean fluorescence intensity of T lymphocytes as 1.0.

The advanced technology of the WPC channel also allows separating suspected malignant cells from healthy ones. This applies to neoplastic lymphocytes with their readily permeated membranes, meaning they will give off clearly higher fluorescence signals (Fig. 4).

The WDF and WPC channels create the whole picture together

The differences in reagents and modes of action between the WDF and WPC channels generate several pieces of information complementing each other. While the lysing reagents in the WDF channel act more gently and retain the intracellular structure of the WBC, the fluorescence reagents of the WPC channel target different intracellular organelles. This explains why a native lymphocyte looks smaller after treatment with WDF reagents than when treated with WPC reagents (Fig. 5a). However, when a lymphocyte is activated, it has a higher cytoplasmic reactivity and therefore it appears to be bigger when stained with the WDF reagents than it does with the WPC ones (Fig. 5b). A stem cell shrinks when treated with either channels' reagents, but its nucleus is stained differently (Fig. 5c). Finally, malignant cells, due to their abnormal membrane composition, allow greater access to their DNA in the WPC channel (Fig. 5d).

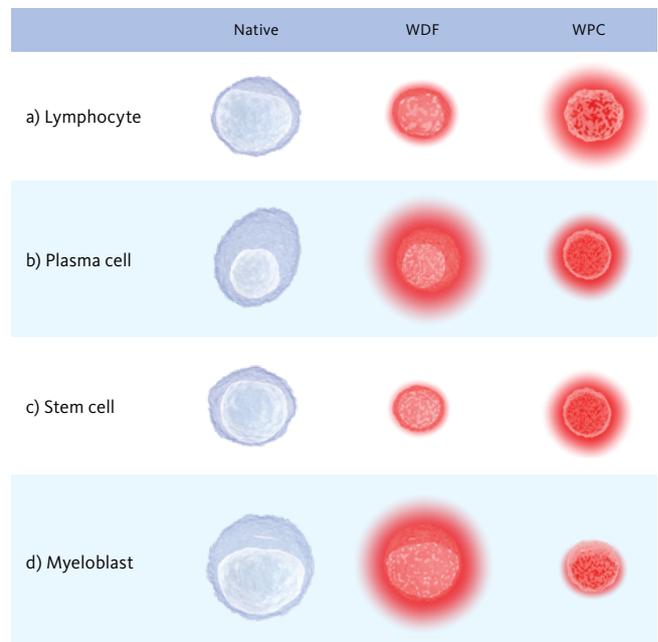


Fig. 5 Differences in the staining mechanisms between the WDF and WPC channel reagents allow separating mature normal cells (lymphocyte; a), activated cells (plasma cell; b), precursor cells (stem cell; c), and malignant cells (myeloblast; d) from each other.

Fig. 4 Subtypes of WBC differentiated by the WPC channel. Populations of T lymphocytes, abnormal lymphoid cells, monocytes and neutrophils are separated after the labelling of their DNA and depending on their intracellular complexity and size. Adapted from Kawauchi et al. [4].

Combining information from both channels establishes a workflow within the Sysmex analysers that optimises the detection of potentially reactive cells and the exclusion of suspected malignant ones [5]. This aids in

a) investigating the immune system response

Inflammation and infection cases can be more easily evaluated through the 'Extended Inflammation Parameters'. These parameters quantify activated lymphocytes, immature granulocytes, and neutrophil activation status. You can find more information in the white paper 'Novel haematological parameters for investigation of the immune system response'.

b) differentiating mature from immature cells

Immature cells such as stem cells can be separated due to their medium size (medium FSC), low granularity (low SSC), and relatively low fluorescence intensity (low – medium SFL). Moreover, since their membrane composition differs from other cells with a comparable size and complexity (like NRBC), it is possible to accurately differentiate them.

Conclusion

Sysmex's advanced technology of fluorescence flow cytometry with its specialised reagents ventures beyond the scope of morphological assessment. It permits to collect much more information from the cell, including its functionality. The combined measurements from the WDF and WPC channels provide additional information about an activation of the immune response as well as the potential presence of immature and/or malignant cells.

References

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