Platelet Detection in Unaltered Whole Blood

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Abstract Platelets are non-nucleated cellular fragments circulating in the peripheral blood, derived from megakaryocytes. They are critical in the maintenance of hemostasis. Alterations in their function are associated with a range of clinical conditions. Using flow cytometry, light scatter signals from platelets typically overlap signals from red blood cells (RBC). Since RBCs outnumber platelets by more than an order of magnitude in whole blood, platelets are typically studied by depleting samples of RBCs by centrifugation methods or selective RBC lysis. These sample preparation procedures require manipulations that may affect platelet health and function, and result in loss of cells. Additionally, lysed samples require labeling of the platelets with a fluorescent marker to adequately resolve the platelets from lysis debris and noise. We present a novel technique for resolving white blood cells (WBC) and platelets from RBCs in whole blood using orthogonal light scatter from 2 lasers. This method exploits the differences in chromatic light-scattering signals between RBCs and platelets and WBCs. Hemoglobin readily absorbs 405 nm laser light, reducing the scatter signal from RBCs relative to WBCs and platelets, resulting in a reproducible scatter pattern when analyzing human whole blood using both blue (488 nm) and violet (405 nm) side scatter. Within this pattern, a distinct platelet population can be discerned in unaltered whole blood, diluted in buffer. Combining this approach with the large dynamic range of sample input rates afforded by acoustic cytometry, allows great flexibility in determining optimal dilution and sample collection rates for minimal coincidence and rapid analysis.

New Methods for No-Lyse No-Wash Whole Blood Detection of Platelets: By exploiting reduced 405 nm side scatter signals due to hemoglobin absorption, platelets can be distinguished from RBCs and WBCs on a 405 nm SSC vs. 488 nm SSC bivariate plot in diluted unprocessed whole blood.

Method 1 demonstrates the basic no-lyse-no-wash concept using differential light scatter to identify RBC, WBC, and platelet populations, separate from each other and from instrument background/noise. This method does not require labeling of platelets for identification. (Figure 1)

Method 2 demonstrates the no-lyse-no-wash protocol with the addition of a CD41-PE antibody conjugate and dual FSC/SSC threshold, to demonstrate optimal dilution of labeled whole blood into buffer for minimal coincidence. (Figure 2) Changing to a fluorescence threshold using phycoerythrin (PE) anti-CD41 antibody has the effect of eliminating the non-fluorescent events from the collection. As platelets are smaller than either RBCs or WBCs, a more narrow Window Extension (WE) setting was applied for greater resolution of the small platelet particles. (Figure 3)

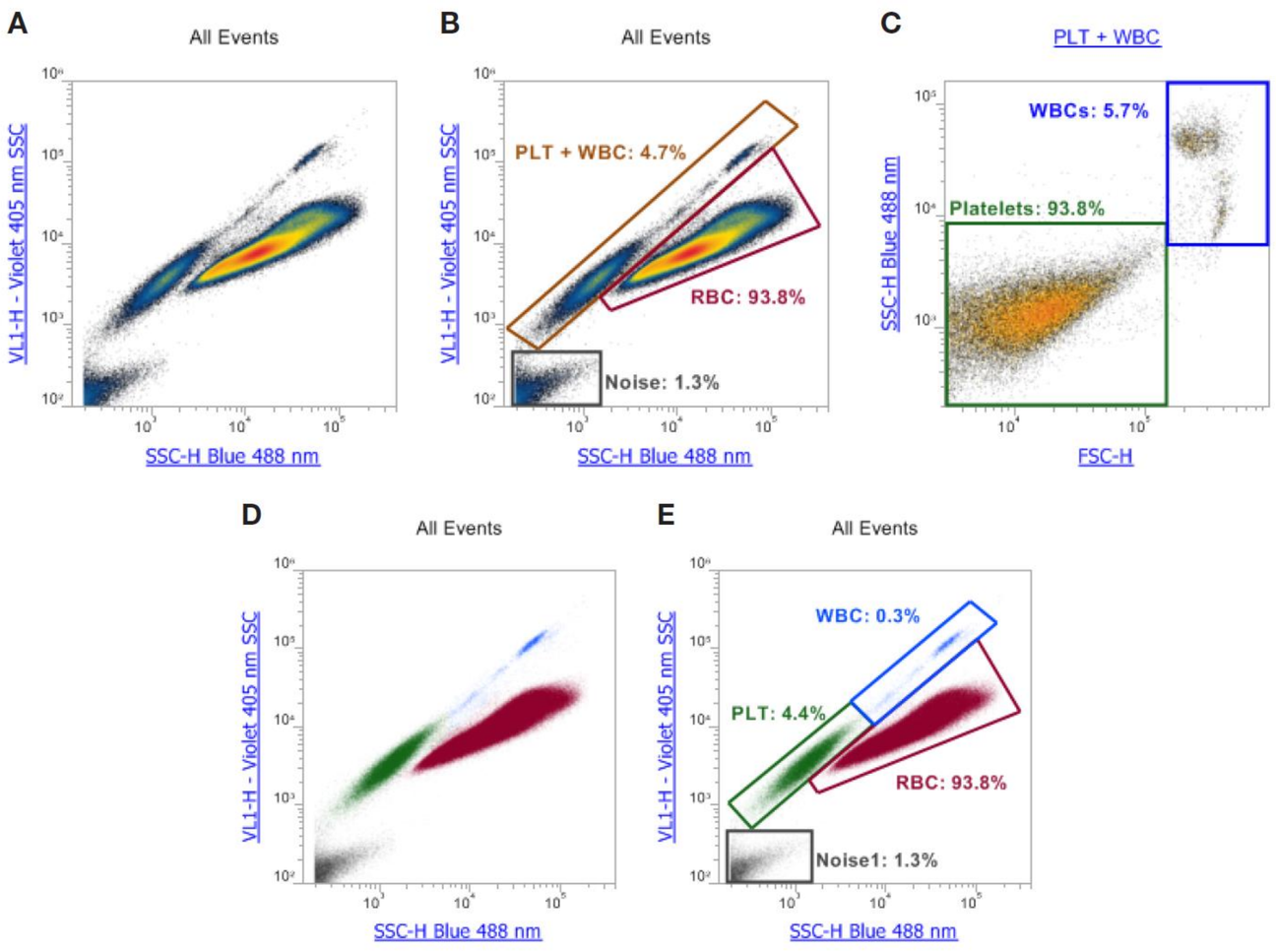

Figure 1. Dual laser blue 488 nm and violet 405 nm laser SSC with intact whole blood without antibody labeling of platelet population (no-lyse/no-wash).

(A, B) RBCs, WBCs, and platelets are separated on the basis of light scatter only by using a combination of blue and violet laser SSC analysis. Hemoglobin in RBCs readily absorbs light at 405 nm, shifting the RBC population to the right by reducing the SSC for RBCs in the violet SSC channel relative to leukocytes and platelets. A dual FSC and SSC threshold is set low enough to show instrument noise, ensuring the full platelet population is visualized.

(C) Using the gate that includes WBCs and platelets, a standard plot of FSC vs. 488 nm SSC can be used to distinguish the platelet population from the WBCs with regions created around the two populations.

(D) Using color-backgating on the same plot as previously shown in (A), the RBC population is colored red, the platelet population is colored green, and the WBC population is colored blue, while the noise is black. The three main WBC populations of lymphocytes, monocytes, and granulocytes can be visualized.

(E) Placing regions around the RBC, WBC, and platelet populations show the dominant cell type in whole blood is the RBC, while the WBC and the platelets are relatively rare events.

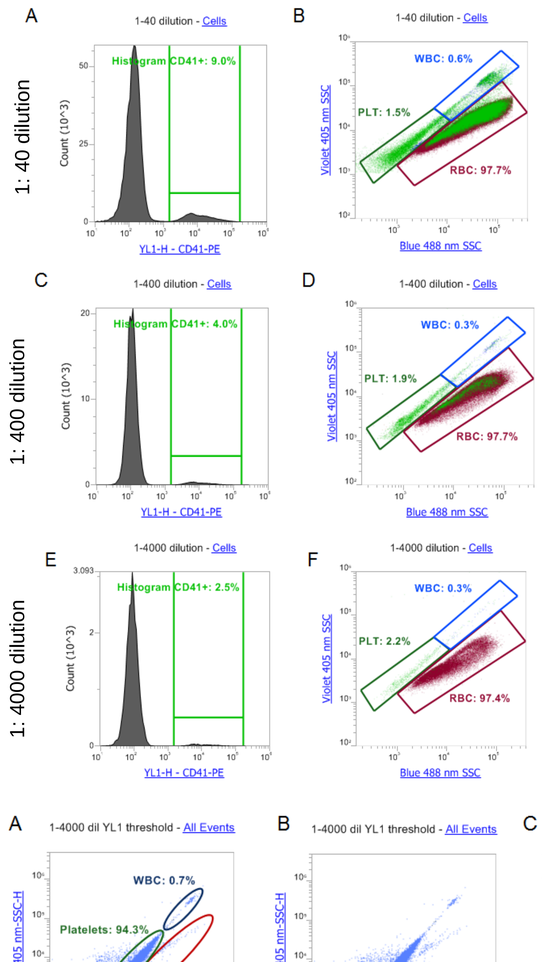

Figure 2. Platelet detection with intact whole blood: reducing coincidence through dilution. Using a dual SSC + FSC threshold, three different dilutions of whole blood into buffer were collected:
(A,B) 40-fold, **(C,D)** 400-fold, **(E,F)** 4,000-fold. All plots use a gate around the total cell population to eliminate the noise events. The PE positive events identified in the histogram and backgated on the scatter plots (light green) reveal significant coincident events with the 40-fold and 400-fold dilutions. The single-parameter CD41 histogram plot thus over-reports the percent CD41 positive at these lower dilutions. At the 4,000-fold dilution, the results demonstrate minimal coincidence, representing the optimal dilution. The CD41 labeling also confirms the position of the platelet population in the scatter profile.

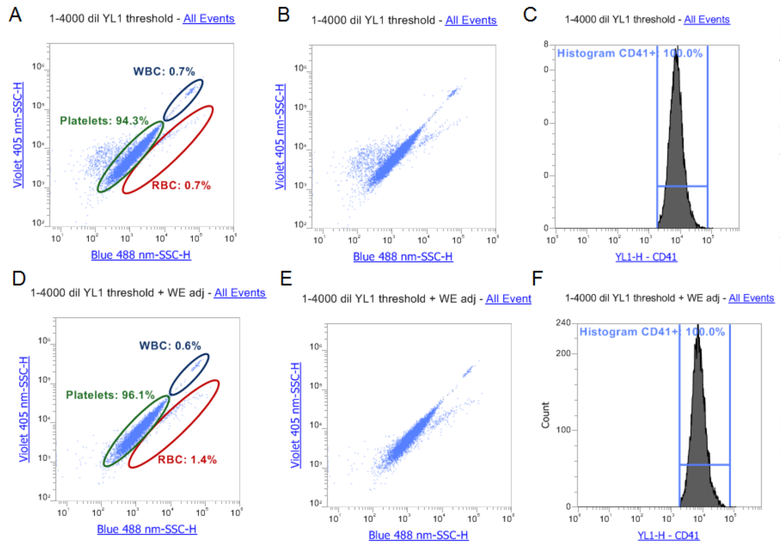

Figure 3. Platelet detection with unlysed whole blood: use of a fluorescence threshold and adjustment of Windows Extension.

Plots A, B, C:
Applying a fluorescence threshold to the 4,000-fold dilution of labeled whole blood eliminates all non-fluorescence events.

Plots D, E,F:
Narrowing the WE setting further eliminates coincident events.

Conclusions

- When analyzing unlysed whole blood samples, platelets can be effectively identified by light scatter alone with the Attune NxT Flow Cytometer using acoustic-assisted hydrodynamic focusing and dual 405/488 nm laser excitation. RBCs can be shifted away from the platelet population due to the strong absorption of hemoglobin with violet 405 nm excitation. Distinct populations of RBC, WBC, and platelet can be identified in whole blood by light scatter alone. The optimal dilution of the labeled whole blood at 4,000-fold dilution demonstrates minimal coincidence.
- The use of CD41-PE labeling with a fluorescence threshold in the whole blood assay confirms the population defined by light scatter is indeed composed of CD41+ platelets. A minor population of apparent CD41+ events is detected outside of the platelet region; by reducing the pulse width by applying a more narrow Window Extension setting further enables accurate detection of platelets by reducing coincidence detection.
- The ability to distinguish the platelet population in unlysed whole blood without sample manipulation beyond dilution presents an improved method for the study of platelets. The high speed collection rates of the Attune NxT Flow Cytometer enables collection of very dilute samples in reasonable collection times.