Individual Red Cell Light Absorption for Cell Type Discrimination

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Introduction

A new single channel, single reagent flow cytometric method identifies RBCs, WBCs, and PLTs without RBC lysis, or RBC coincidence signals overlapping WBCs as occurs in hematology analyzers due to high RBC sampling rates. Samples are analyzed using 406 nm laser illumination. Hemoglobin light absorption in individual RBCs is directly measured by collecting a 17° cone that captures almost all RBC scattered light, thus registering only absorption. WBCs and PLTs are unabsorbing at 406 nm. The method works even at high RBC concentrations. It is not affected by large PLTs, microcytic or hypochromic RBCs; nor by RBC nucleation, and thus useful for avian and reptilian samples. Other single channel methods work only for human samples. For the first time, directly measured individual RBC hemoglobin absorption enables automated cell type discrimination in human, other mammalian, avian, and reptilian samples.

Methods

Human and avian blood samples were diluted in a medium that spheres and fixes RBCs. Human RBC fractions were prepared by passing whole blood through Pall Acrodisc WBC filters and collecting the WBC depleted fractions. WBC fractions were prepared by backflushing used filters with NH_4Cl to lyse residual RBCs. Human whole blood and RBC fraction samples were diluted 50-fold to demonstrate insensitivity to RBC coincidence. Human WBC samples were undiluted. Avian samples were diluted 1000-fold due to limited sample. Samples were run on a modified commercial hematology analyzer; light source replaced by a 406 nm laser, standard diluent replaced by sphering and fixing diluent. Two measurements were made on each cell; light scattered over 17° and orthogonally (80-100°). 20000 cells were analyzed for each sample. Data was collected in FCS format and displayed as right angle vs. 17° (absorption) plots.

Results

The results are presented in Figures 1-8.
Figures 1A-C are graphs of absorption and scatter for sphered RBCs over the range of size HGB concentration usually encountered, based on Mie Scattering Theory. They show that absorption signals at 406 nm are comparable in size to typically used scatter signals at 670 nm.
Figures 2A-C are side scatter/absorption plots for RBC preparations in various media. The PBS plit is at higher absorption gain to show where PLTs appear.
Figure 3 is a plot for WBC preparation showing where the WBCs appear.
Figure 4A-C are plots of overlays of WBC and RBC preparations in various media to show that the WBC and RBC populations are distinct.
Figures 5A-C are plots of whole blood sample run at increasing concentration/RBC coincidence level to that even at high coincidence RBCs and WBCs are distinct.
Figure 6 is a plot for a bird whole blood sample to show that method distinguieshes between NRBCs and WBCs, and between thrombocytes and lymphocytes.
Figure 7 includes graphs of 60 fL PLT and 15 fL/23g/dL RBC scatter vs. angle as well as the absoprtion signal value for RBC based on Mie Scattering Theory to show that even very microcytic/hypochromic human RBCs have much larger signals than even very large human PLTs.
Figures 8 A-B are graphs of absorption channel mean vs. MCH and absoprtion channel mean vs. calculated absorption cross section for 8 whole blood samples run in a sphering medium.

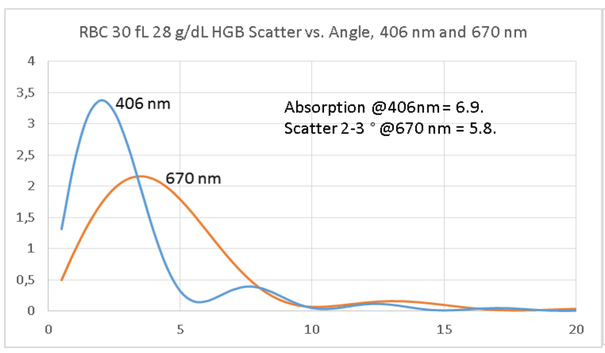


Figure 1A

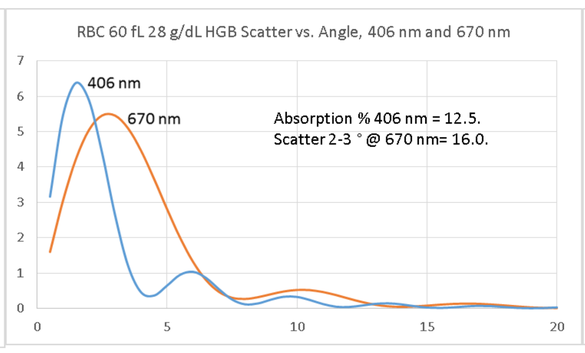


Figure 1B

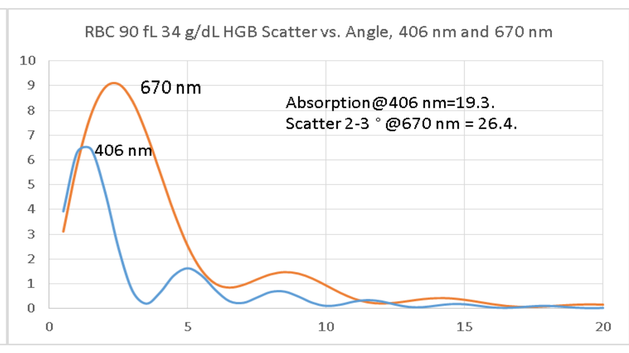


Figure 1C

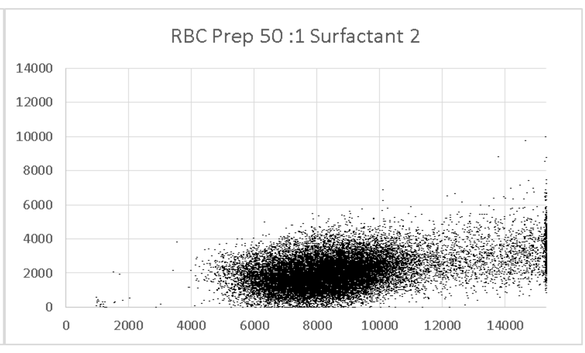


Figure 2A

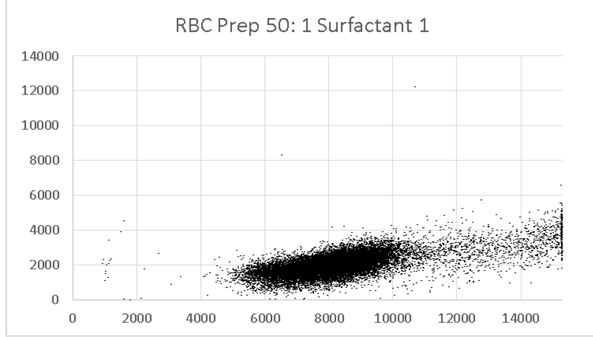
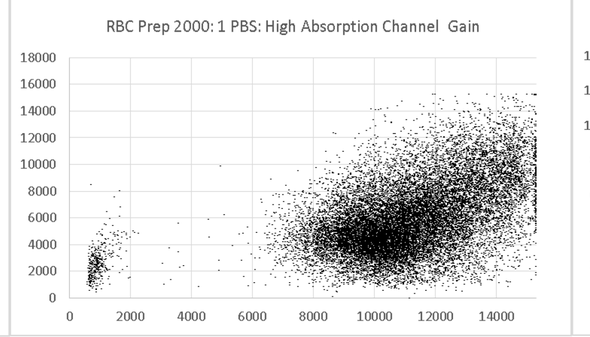


Figure 2B



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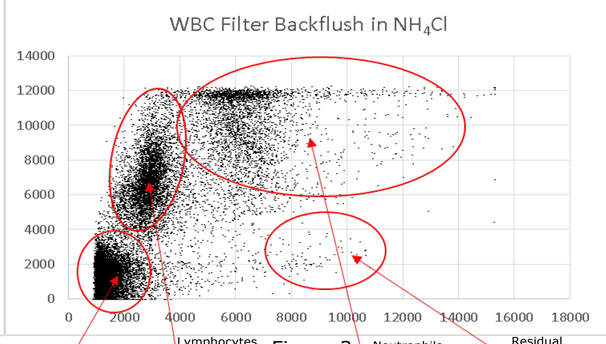


Figure 3

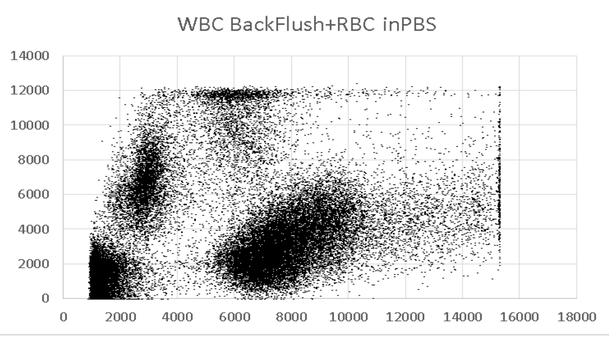


Figure 4A

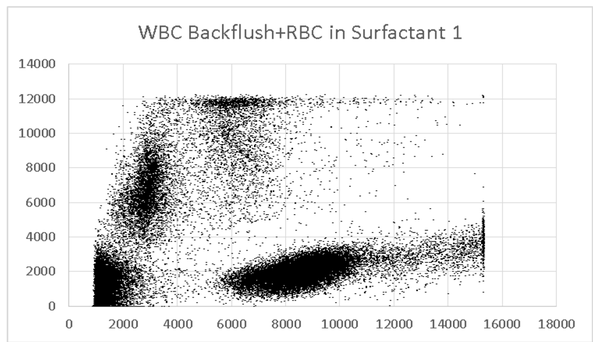
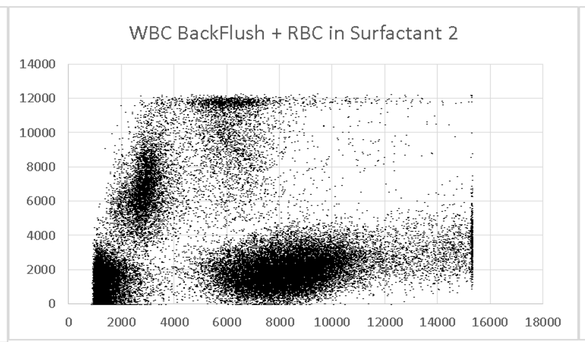


Figure 4B



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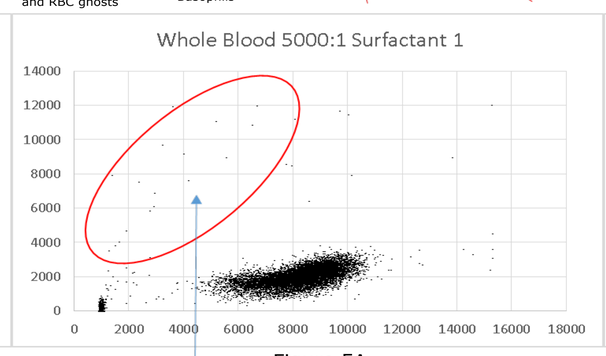


Figure 5A

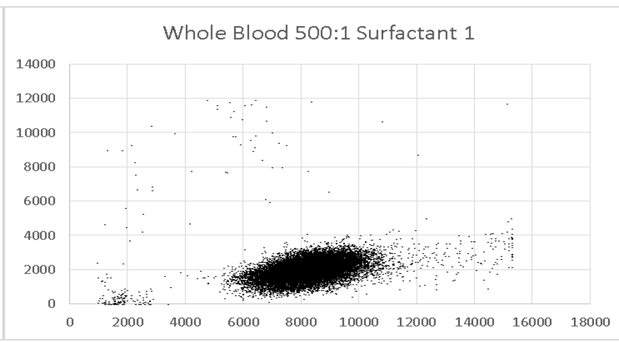
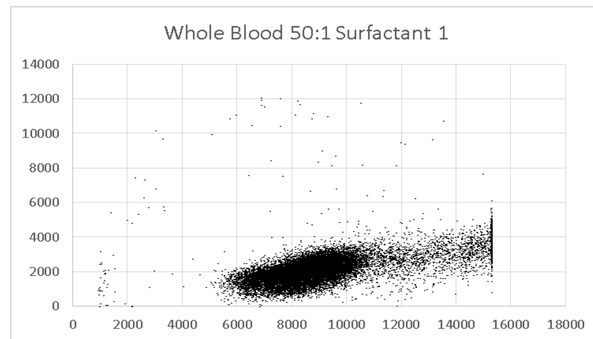


Figure 5B



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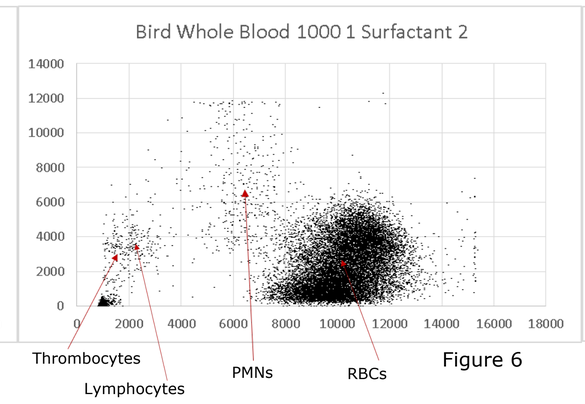
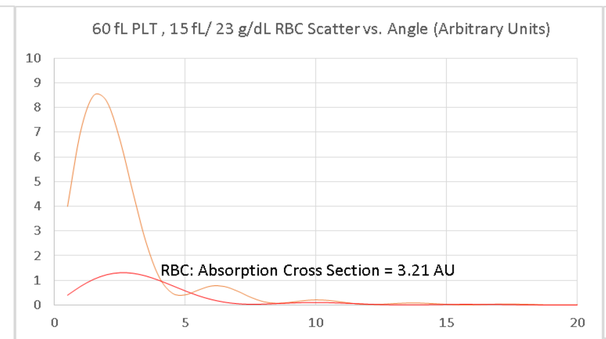


Figure 6



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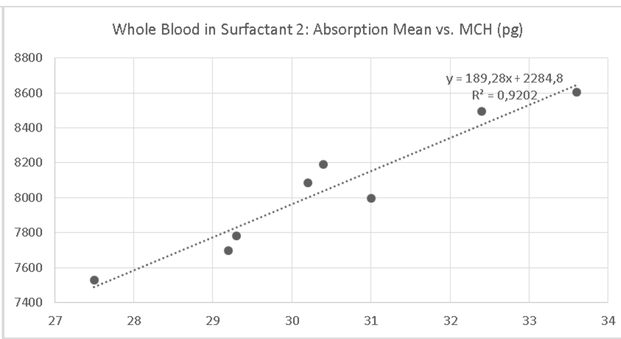

Figure 8A

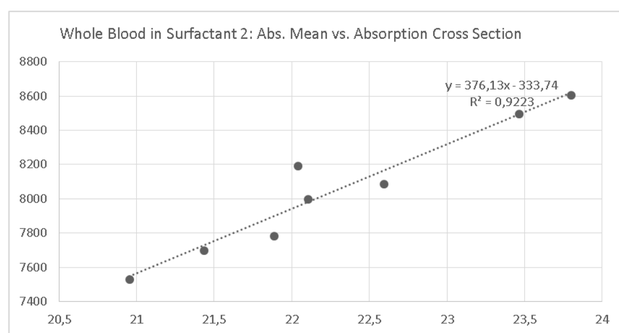

Figure 8B

Conclusion

406 nm absorption by individual RBCs generates robust signals, distinct from WBC and PLT signals on right angle vs. absorption plots. This discrimination method applies even at very high RBC concentrations/coincidence levels, and is therefore suitable for automated hematology analyzers requiring high sampling rates. The method also works for birds and reptiles, with large nucleated RBCs and large thrombocytes. The method discriminates even very microcytic/hypochromic RBCs from even very large PLTs. The method allows for the determination of MCH and HGB from 15 fL to 300 fL and 23 g/dL to 36.5 g/dL< covering the ranges encountered in chordates.

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