WHITE PAPER

anvajo

Complete blood count based on **digital holographic microscopy:**

A Label-Free Approach for Rapid Blood Cell Analysis at the Point of Care

Complete Blood Count (CBC) analysis is a fundamental diagnostic tool for assessing the cellular composition of blood. Digital Holographic Microscopy (DHM) offers a label-free imaging approach that enables rapid blood cell detection, classification, and volume estimation based on intrinsic optical and morphological properties. In this study, peripheral blood samples were analyzed using an anvajo DHM-based system combined with advanced machine learning algorithms. Leukocyte-containing fractions were prepared by erythrocyte lysis and imaged without labeling, while diluted whole-blood samples were used for erythrocyte and thrombocyte analysis. Reconstructed holographic data enabled automated cell detection within less than 30 seconds after acquisition. Unsupervised machine learning revealed distinct clustering of the five major leukocyte subtypes, while erythrocytes and thrombocytes were differentiated based on characteristic DHM-derived optical parameters. Preliminary volume measurements of red blood cells and thrombocytes showed strong agreement with expected physiological distributions. These findings demonstrate the potential of DHM as a rapid, reagent-efficient, and label-free technology for point-of-care CBC analysis.

Introduction

CBC analysis provides essential information on the cellular composition of blood. Conventional hematology workflows typically rely on complex instrumentation, reagent-based sample preparation, and centralized laboratory infrastructure. These requirements can limit flexibility in decentralized or point-of-care settings, where rapid turnaround times, simplified handling, and reduced consumable use are increasingly important.

DHM offers a promising alternative for blood cell analysis. As a label-free imaging technology, it captures quantitative optical information from individual cells without the need for staining or fluorescent markers. By combining phase and amplitude information with digital image reconstruction, DHM enables the analysis of morphological and optical cell properties, including size, shape, refractive characteristics, and volume-related parameters.

Previous studies have demonstrated the applicability of holographic and quantitative phase imaging for hematological analysis. Vercruysse et al. showed the feasibility of lens-free

holographic microscopy for leukocyte differentiation, while Yi et al. demonstrated the value of quantitative phase imaging for red blood cell characterization. Building on these principles, anvajo has developed a DHM-based analytical approach that combines label-free imaging with advanced machine learning to support rapid classification and characterization of blood cells.

Sample Preparation and Workflow

Peripheral blood samples were collected via finger-prick. For white blood cell (WBC) analysis, erythrocytes were removed by lysis, resulting in a leukocyte-containing sample fraction. This fraction was imaged using the anvajo DHM-based system.

For red blood cell (RBC) and thrombocyte analysis, native whole blood was diluted at a ratio of 1:500 and subsequently imaged using DHM.

After hologram acquisition, the images were digitally reconstructed and processed to identify individual cells and to proceed with volume estimation of erythrocytes and thrombocytes.

To evaluate the differentiation of leukocyte subtypes, individual cells were analyzed using unsupervised machine learning. In this approach, cells are not assigned to predefined categories during training. Instead, cells with similar morphological and optical properties are automatically grouped within a multidimensional feature space.

Results

Leukocyte analysis showed a clear separation of five white blood cell groups based on DHM-derived parameters. Additionally, obtained phase signatures were consistent with previously reported data from the literature[3], supporting the reliability of the measurements and demonstrating the potential of DHM combined with machine learning for label-free white blood cell differentiation.

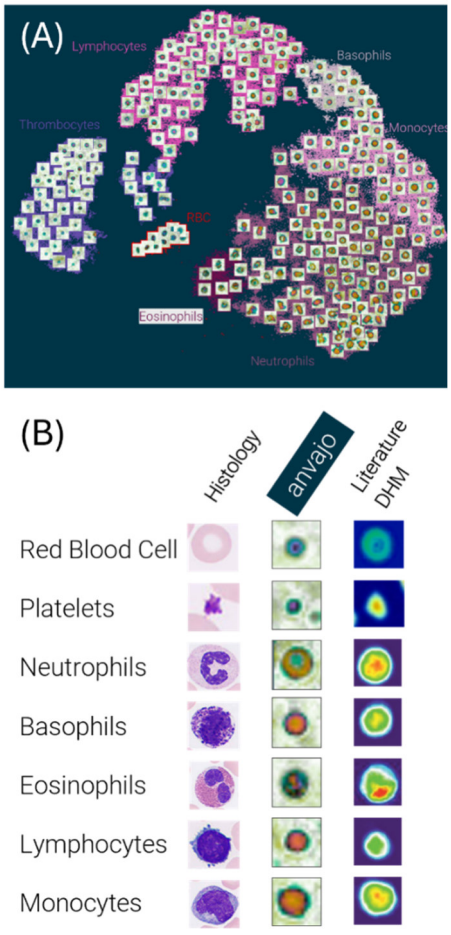

Figure 1. (A) Blood cell clusters obtained through unsupervised machine learning analysis. (B) Blood cell types observed using conventional histology and DHM imaging[3].

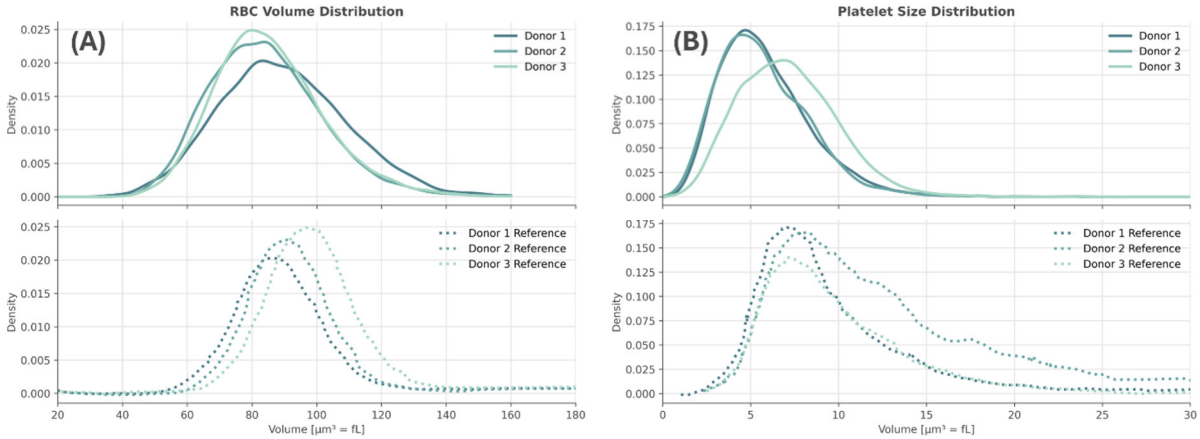

Figure 2. (A) Red blood cell and (B) platelet volume distributions measured in three healthy donors and compared with corresponding reference distributions.

For erythrocytes and thrombocytes, volume distributions were measured across samples from three healthy donors. The preliminary results showed strong agreement with expected physiological characteristics. In particular, the typical skewed volume distribution of thrombocytes was well reflected by the DHM-based measurement approach.

Discussion

The results indicate that DHM can provide a rapid approach for DHM based blood cell analysis. This technology enables simultaneous access to morphological, optical, and volume-related parameters, creating a rich basis for automated cell classification.

The combination of DHM with machine learning is particularly relevant for point-of-care diagnostics. By reducing sample preparation requirements and avoiding labeling steps, the workflow can be simplified compared with conventional methods.

A key enabler of this performance is the locally adaptive focusing method, since cells are located at different axial positions within the sample. This allows the system to extract additional three-dimensional information and improves the quality of cell-specific measurements.

Thus, enabling image reconstruction, cell detection, and downstream analysis within a short time frame further supports the potential of this technology for decentralized diagnostic applications.

However, study data represents just the beginning of transferring DHM into clinical applications for blood cell analysis. Further research and refinement of image data and recording system are required to obtain even more precise results and provide statistically robust data required for clinical diagnostics.

Conclusion

Digital Holographic Microscopy represents a promising label-free technology for rapid Complete Blood Count analysis. The anvajo DHM-based platform combines quantitative phase imaging, digital reconstruction, adaptive focusing, and machine learning to enable fast and accurate characterization of blood cells.

The demonstrated differentiation of the five major leukocyte subtypes, together with volume estimation of erythrocytes and thrombocytes, highlights the potential of this approach for point-of-care hematology. By providing rapid analysis without extensive sample preparation or labeling, DHM may contribute to more accessible, efficient, and scalable CBC testing in decentralized healthcare environments.

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2. Yi F, Moon I, Javidi B, Boss D, Marquet P. Automated segmentation of multiple red blood cells with digital holographic microscopy. J Biomed Opt. 2013 Feb;18(2):26006. doi: 10.1117/1.JBO.18.2.026006. PMID: 23370481.
3. Ugele M, Weniger M, Stanzel M, Bassler M, Krause SW, Friedrich O, Hayden O, Richter L. Label-Free High-Throughput Leukemia Detection by Holographic Microscopy. Adv Sci (Weinh). 2018 Oct 11;5(12):1800761. doi: 10.1002/advs.201800761. PMID: 30581697; PMCID: PMC6299719.