Human peripheral blood mononuclear cell scattering database

David Dannhauser*[a], Paolo A. Netti[a,b], Filippo Causa[a]
[a]Interdisciplinary Research Centre on Biomaterials (CRIB) and Dipartimento di Ingegneria Chimica, dei Materiali e della Produzione Industriale, University of Naples "Federico II", 80125 Naples, Italy;
[b]Center for Advanced Biomaterials for Healthcare@CRIB, Istituto Italiano di Tecnologia, 80125 Naples, Italy; *david.dannhauser@unina.it

ABSTRACT

The human body contains a wide variety of cell types, each with distinct physical and functional characteristics. Capturing this cellular diversity is critical for biomedical research and diagnostics, yet traditional classification methods often rely on labeling, which can be time-consuming and costly. In this study, we present a label-free technique that uses static light scattering to identify and classify human peripheral blood mononuclear cells (PBMCs). These cells, easily obtained from blood samples, offer a non-invasive means of assessing health and disease states. We introduce a microfluidic system that channels single cells through a light-scattering measurement zone, capturing unique optical patterns from each cell. These scattering signatures are then analyzed using deep learning models trained on human blood cell data. Our approach is self-supervised, eliminating the need for large, annotated datasets and allowing for accurate classification without prior labeling. Compared to flow cytometry, our system reduces complexity, lowers costs, and requires fewer sample cells. Importantly, cells remain viable for further analysis or therapeutic use. This innovative, label-free method offers a practical and efficient tool for studying cell heterogeneity and holds strong potential for use in clinical diagnostics and personalized healthcare applications.

Keywords: Single Cell, Database, Static Light Scattering, Microfluidics, Circulating Tumor Cells, Deep Learning, PBMCs

1. INTRODUCTION

Human cells exhibit diverse biophysical properties -such as size, structure, and function- that reflect their inherent characteristics and specialized roles within the body. This intrinsic heterogeneity arises from differences in cellular lineage, physiological state, and environmental exposure, and it plays a critical role in health, disease progression, and immune response [1]. Accurately characterizing this diversity is essential for numerous biomedical applications, including disease diagnosis, therapeutic monitoring, and the advancement of personalized medicine [2]. However, conventional methods for cell identification typically rely on molecular labels or stains, which can be time-consuming, costly, and may alter the natural state of the cells. As a result, there is an increasing demand for robust, label-free techniques capable of classifying cells based solely on their physical and optical properties.

One promising approach for label-free cellular analysis is light scattering, which leverages the interaction of light with intracellular structures to infer morphological and structural features [3]. Light scattering methods have shown strong potential in identifying and characterizing various cell types -including circulating tumor cells and immune cells- under a range of physiological and pathological conditions. Among these methods, static light scattering stands out as a high-throughput, non-invasive technique capable of capturing detailed optical signatures that encode the physical architecture of individual cells [4].

In this work, we focus on the application of static light scattering to analyze cells circulating in peripheral blood, with particular emphasis on human peripheral blood mononuclear cells (PBMCs). PBMCs, comprising lymphocytes and monocytes, are easily isolated and serve as valuable models for studying immune system function and identifying disease-associated biomarkers. Notably, PBMCs are widely used in "liquid biopsy" applications, offering a minimally invasive means of assessing systemic health and detecting early signs of disease [5]. We present a scattering image database of human PBMCs, acquired using a microfluidic-based static light scattering apparatus. In this system, small-angle light scattered by individual cells flowing through a microfluidic channel are recorded, producing unique optical

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profiles that reflect both cellular morphology and internal structure [6]. These scattering signatures are then analyzed using deep neural networks trained on peripheral blood cell datasets to accurately classify cell types [7]. A key advantage of our approach is the use of a self-supervised learning framework, which minimizes the need for extensive labeled datasets and enhances generalizability across diverse sample types. However, a cell class database is needed, which uniforms the recorded datasets.

2. MATERIAL AND METHODS

PBMCs were isolated from healthy donors following informed consent, in compliance with all relevant ethical guidelines and regulations. PBMCs were separated using standard density gradient centrifugation, followed by negative selection with antibody-conjugated magnetic beads to isolate specific cell populations [8,10].

Single-cell biophysical properties were measured using a custom microfluidic device composed of a 3D-printed structural frame that combines a glass capillary with a channel. In fact, the pressure-driven flow aligns the cells in the circular shaped glass capillary. This is followed by a square-shaped readout channel, which preserves cell alignment and optimizes optical access (see Fig. 1). The alignment is achieved by using a viscoelastic polyethylene oxide (PEO) solution (MW = 4 MDa), with its properties finely tuned by flow velocity and channel geometry [8,11]. Post-measurement, cells remain viable and can be collected for downstream analysis.

For data acquisition, we employed a small-angle light scattering setup integrated with the microfluidic system [8–11]. As cells traverse the readout channel, they sequentially intercept a collimated 632.8 nm laser beam, producing distinct scattering image. Scattering was recorded over a continuous angular range of 3°–33°, with ~0.1° resolution. Images were captured using a camera sensor (700×700 pixels, 6.5 µm pixel size) with a 4 ms exposure time (see Fig. 1) [8–15].

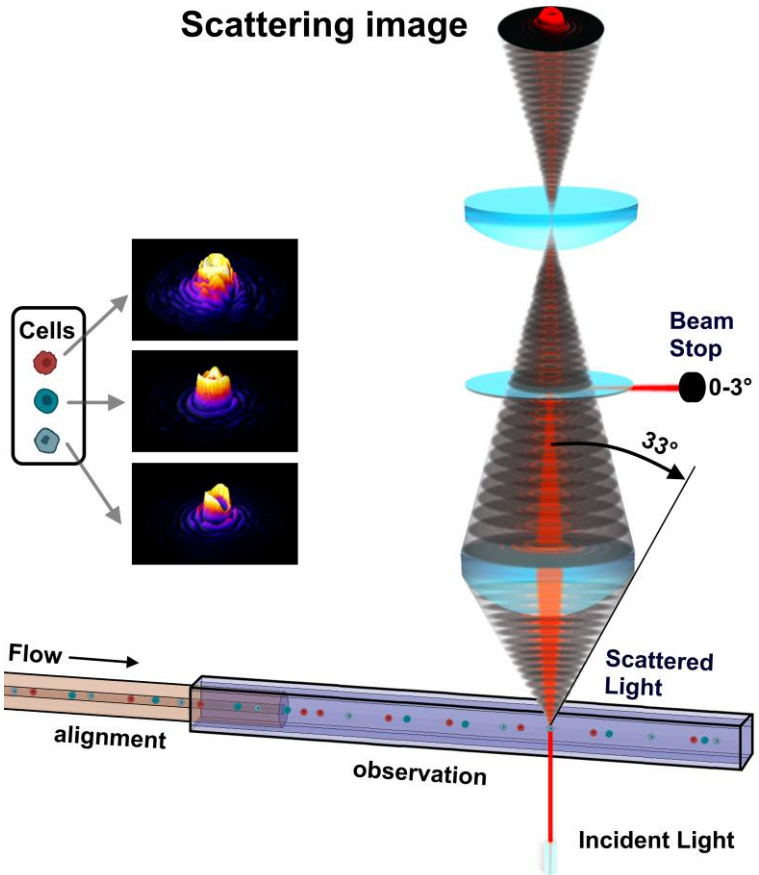

Figure 1. A vertical light beam is directed through a microfluidic device. As individual cells flow through the device and intersect the incident light path, they scatter the light, which is captured by a lens and mapped on a camera sensor, which records the scattering image.

To prepare the data for convolutional neural network (CNN) analysis, the scattering images were pre-processed to standardize and enhance pattern quality. This involved centroid detection and the application of a binary mask that excluded angular regions outside the 3°–33° range. The mask was generated through a pipeline combining iterative 2D Gaussian low-pass filtering, low-light image inversion for spot enhancement, haze reduction, and global thresholding (Fig. 2). The resulting segmented images were then centered, cropped to 650×650 pixels, and resized to 224×224 pixels for the CNN dataset.

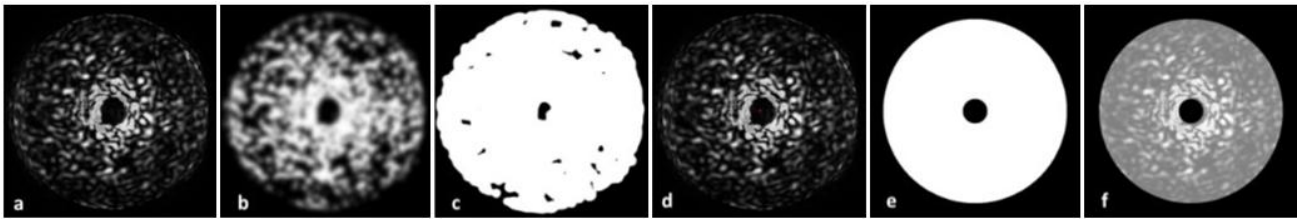

Figure 2. Illustrative scattering image pre-processing: (a) on the raw scattering image, (b) a 2D gaussian low-pass filter (size 15x15 pixel and standard deviation of 6) was iteratively applied four times, followed by a spot-enhancement using a lowlight image enhancement based on inversion of low-light image, applying a haze removal algorithm and an inversion of the enhanced snapshot image. Note that haze thickness at each pixel and the estimated atmospheric light were not used for the image pre-processing. Then, image intensity values were mapped on new values before a global threshold. All these steps are done to merge the separated image components in a unique binary mask (c), to finally perform a segmentation on the raw scattering image. Subsequently, a centroid detection function returns the 0° coordinate of the scattering image (d). The centroid value was used as the center for a donut mask (e) used to perform segmentation on the raw image (f).

3. RESULTS

Over the past decade, we have developed a comprehensive scattering image database of human peripheral blood cells, encompassing all major PBMC subtypes with the exception of dendritic cells (see Fig. 3). In addition to PBMCs, the dataset includes erythrocytes and polystyrene beads. The beads serve both as system calibration standards and as proof-of-concept elements. The primary objective of this database is to provide well-characterized datasets for distinct PBMC classes that can be shared with the scientific community. Each cell type, including its subpopulations, was collected from a minimum of four different healthy donors to capture biological variability and enhance dataset robustness.

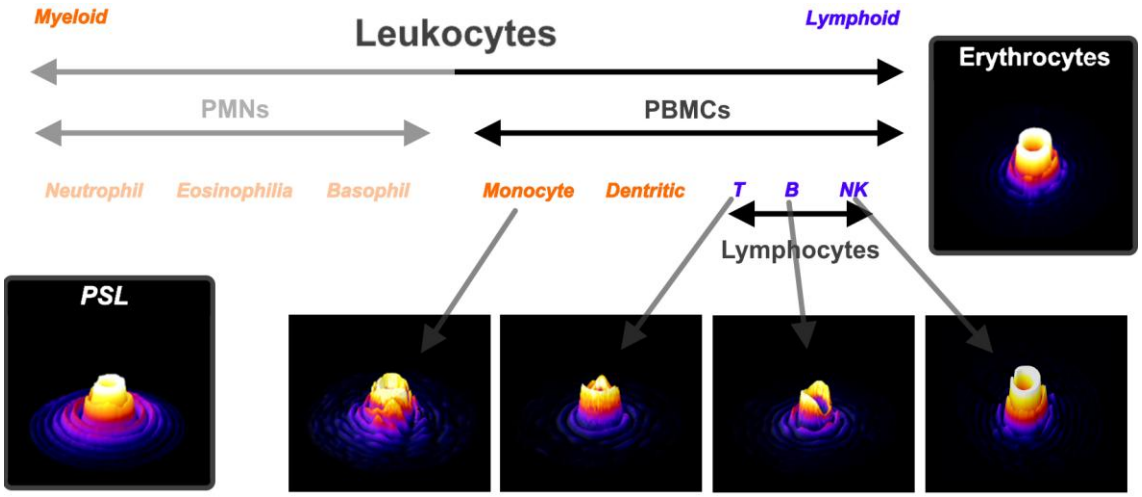

Figure 3. PBMC classification and general Leukocyte overview with 3D surface plots of cells. Leukocytes are divided into myeloid and lymphoid cells based on their origin or on the morphology of their nucleus (mononuclear or polynuclear) and the presence or absence of granules in their cytoplasm, which results in two main cell types (peripheral blood mononuclear cells (PBMCs) and polymorphonuclear leukocytes (PMNs). We also illustrate typical scattering images of erythrocytes and polystyrene beads.

Scattering images were preprocessed by centering on the scattering pattern and cropping peripheral regions to highlight relevant features (see Fig. 2). To maintain data quality, a CNN-based image classifier was implemented to automatically identify and exclude cellular debris and aggregates, ensuring the retention of single-cell images. As of now, the curated database comprises over 10,000 scattering images. This dataset will be made publicly available via the GitHub platform to support further research and community collaboration [16].

4. CONCLUSION

Compared to conventional flow cytometry, our approach offers several notable advantages. It reduces both instrumentation complexity and operational costs, while enabling accurate cell classification using smaller sample sizes, thereby enhancing overall efficiency and accessibility. By eliminating the need for labor-intensive and resource-demanding labeling procedures, our label-free analysis streamlines the workflow and minimizes sample preparation time. Additionally, the method preserves cell viability, allowing for the analysis of living cells in suspension, facilitating downstream applications such as functional assays or therapeutic interventions. Our research group is actively expanding the database and is committed to making it publicly accessible to support and accelerate further research in the field.

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