WHITE PAPER

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Digital holographic microscopy - **the portable key to novel applications in diagnostics?**

Modern clinical diagnostics relies heavily on optical imaging and measurement technologies such as brightfield and fluorescence microscopy. While these methods have significantly advanced laboratory medicine, they remain associated with substantial limitations including staining dependency, subjective interpretation, high operational complexity, limited automation, and restricted preservation of viable cells. These challenges are particularly relevant in applications requiring rapid, quantitative, and reproducible analysis of biological fluids and cellular samples.

Digital Holographic Microscopy (DHM) represents an innovative and transformative approach to diagnostic imaging by enabling 3D label-free, quantitative, and computationally enhanced analysis of cells and particles in their native state. Unlike conventional optical systems, DHM captures holographic interference patterns containing both amplitude and phase information, which are reconstructed computationally into high-resolution images. This approach eliminates the need for staining while preserving live-cell integrity and enabling fully automated workflows supported by artificial intelligence.

Recent feasibility and proof-of-concept studies as shown below demonstrate the broad applicability of DHM across clinical diagnostics. The technology has shown strong performance in complete blood count analysis, rare cell detection including circulating tumor cells (CTCs), cerebrospinal fluid (CSF) cytology, urine sediment analysis, and cell viability assessment. In multiple studies, DHM-based systems achieved close agreement with established clinical reference methods while offering faster processing, lower sample preparation requirements, and enhanced automation potential.

Importantly, DHM combines compact optical hardware with advanced computational imaging, enabling scalable and portable diagnostic platforms suitable for

both centralized laboratories and point-of-care environments. The integration of machine learning further enhances object recognition, classification accuracy, and reproducibility without requiring extensive operator expertise.

Looking ahead, emerging developments such as nanoparticle-assisted contrast enhancement and multicolor or ultra-high-resolution computational imaging are expected to further expand the diagnostic capabilities of DHM. These innovations position DHM as a promising next-generation platform technology for personalized medicine, decentralized diagnostics, and high-throughput clinical screening across a broad range of biological fluids including blood, urine, cerebrospinal fluid, and other cellular samples.

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Introduction

While modern clinical diagnostics strongly depend on optical imaging systems that include brightfield or fluorescence microscopy, flow cytometry or similar, these technologies still present several limitations impacting accessibility or availability of diagnostic results in acute or point-of-care settings. Still, innovation in the market is progressing slowly. Here, DHM creates a quick and label free alternative providing an important tool in the future for personalized or centralized medicine investigations, spanning analysis of blood, urine, sperm or any other type of cell or particle containing body fluids.

DHM is an imaging technique that captures how light interacts with a sample. Instead of forming an image through lenses, DHM records an interference pattern generated by the interaction of scattered and un-scattered light. This hologram contains intensity and phase information. Using computational reconstruction, the system generates a high-resolution representation of the sample, including structural and morphological details as well as 3D information. This approach enables the analysis of samples without staining in their natural state.

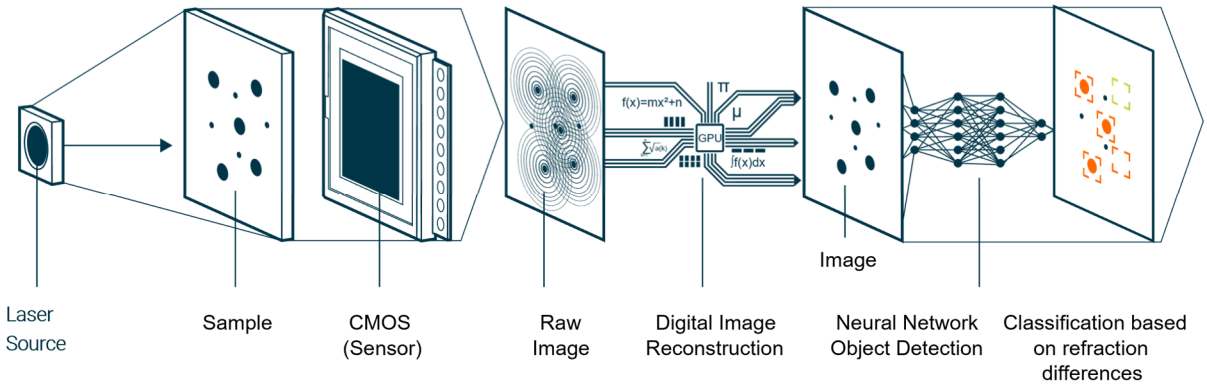

Figure 1. Optical Measurement and Signal Processing Workflow for DHM

Unlike conventional microscopy, where image quality is primarily determined by optical hardware, DHM relies on digital signal processing enabling a hardware module fitting in the palm of your hand plus omitting any moving parts. The computational

imaging approach enables software-defined optimization and AI-based interpretation enabling automated and reproducible analysis without manual interpretation.

Results

Complete blood count

Complete blood count (CBC) is one of the most frequently ordered diagnostic tests in clinical practice. However, current workflows rely on centralized laboratory infrastructure, resulting in delays of 1–24 hours depending on setting. In acute care, emergency medicine, and resource-limited environments, this delay can impact clinical decision-making. Thus, there is a growing need for rapid, decentralized, and cost-efficient point-of-care (PoC) CBC solutions that maintain laboratory-grade accuracy.

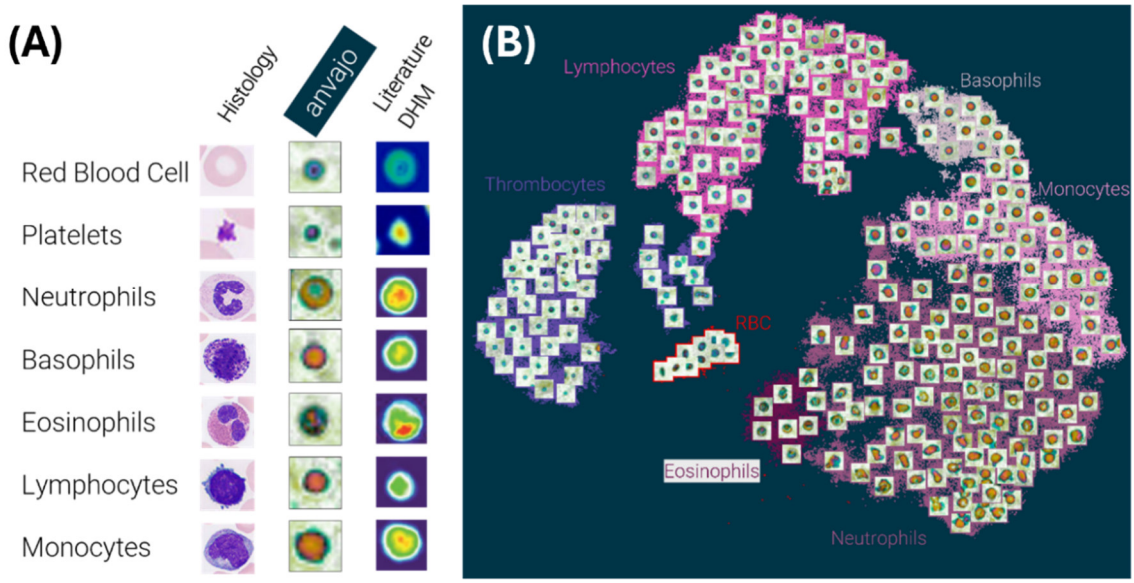

Figure 2. (A) Comparison of light microscopic and DHM derived blood cells and (B) cluster analysis generated with unsupervised learning tools

To prove feasibility of assessing a 5-part differential blood count by using DHM and an image-based analysis pipeline, a study was performed using whole blood samples. Here, while benchmarking against the Sysmex Reference System, DHM showed successful extraction of a **5-part WBC differential** from holographic images including measurement of **RBC and platelet size distributions** showing strong plausibility demonstrating the feasibility of a label-free, image-based point-of-care CBC system capable of producing clinically relevant hematology parameters.

Rare cell detection (circulating tumor cells)

Circulating tumor cells (CTCs) are rare cancer cells shed from primary or metastatic tumors into the bloodstream. Their detection and characterization provide critical insights into metastatic progression, treatment response, and minimal residual disease. Despite their clinical relevance, CTCs remain underutilized in routine oncology due to technical and biological challenges.

Current technologies either prioritize molecular specificity (losing morphology) or morphological imaging (requiring extensive sample preparation and labeling). There is no widely adopted system combining label-free detection, scalability, and quantitative single-cell characterization. Despite advances, a major gap remains in the ability to detect label-free CTCs with high sensitivity, preserve morphological and biophysical cell features, enable high-throughput single-cell analysis without enrichment bias and transition CTC analysis from centralized laboratories to near-patient settings. DHM provides the potential to close this gap while importantly, preserving live-cell integrity for downstream molecular analysis and personalized medicine applications.

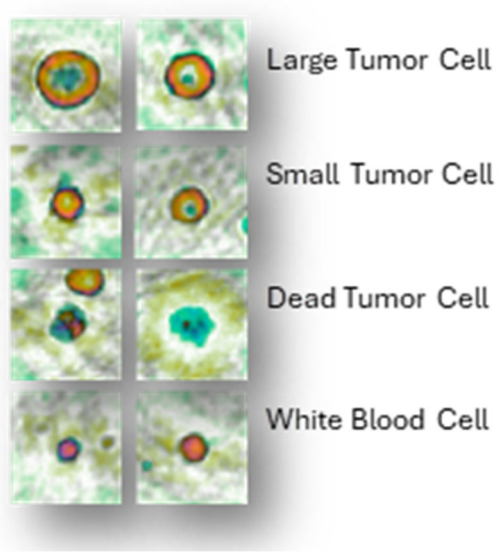

Figure 3.Phase images of different tumor cells and white blood cells acquired during the study.

In a feasibility assessment, a simple neural network was trained on 1.5 million single WBC and 3 different types of cancer cells. Subsequent sample analysis consisting of isolated PBMCs spiked with known and unknown cancer cell types from cell culture proofed **detection capability of cancer cells lower than 1/µl.** Obtained counts were very close to the established clinical reference method, while being much faster by delivering results in seconds and omitting manual correction of automized labeling, thus providing the proof of the detection of rare malign cells amidst healthy blood cells.

Low cellularity biological fluids (cerebrospinal fluid)

Accurate quantification of RBCs and WBCs in cerebrospinal fluid (CSF) is critical for diagnosing neurological conditions such as meningitis, hemorrhage, trauma, and neoplasia. Despite clinical importance, there is a notable lack of automated hematology analyzers specifically optimized for CSF analysis. Most laboratories rely either on manual hemocytometer-based counting—subject to significant operator variability and limited precision—or on blood-optimized automated analyzers, which may suffer from reduced sensitivity, and suboptimal performance at low cell concentrations. This gap underscores the need for dedicated or adapted technologies capable of delivering reliable, reproducible CSF cell counts to support timely and accurate clinical decision-making.

This proof-of-concept study evaluates the analytical performance of a DHM based algorithm on veterinary samples compared to the ADVIA 120 hematology analyzer, a widely accepted laboratory reference standard.

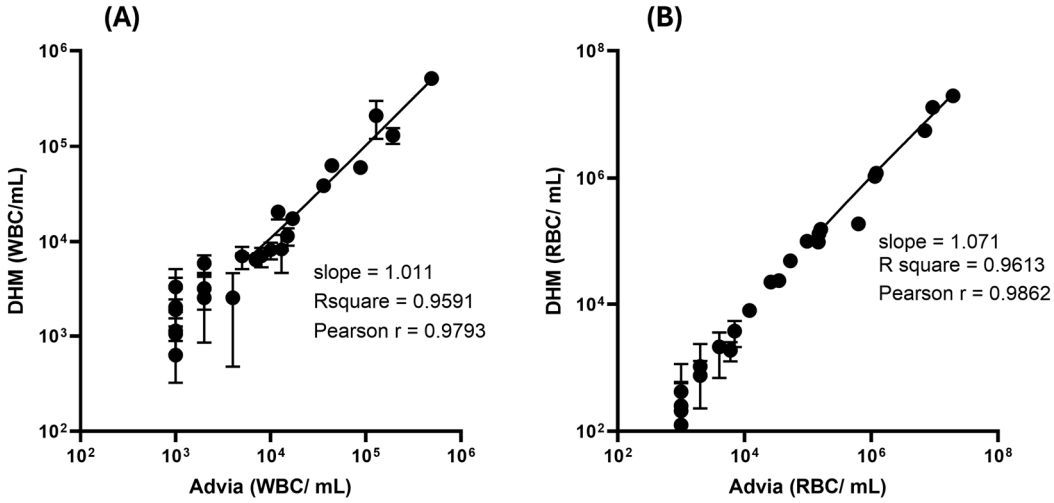

Figure 4. Correlation between DHM and Advia blood cell quantification results for WBC (A) and RBC (B).

Across 30 measurements, DHM demonstrates **strong agreement with ADVIA 120 for both RBC and WBC counts**, with high linearity and excellent correlation, based on low sample volume underscoring DHM as a reliable, rapid, and potentially field-deployable alternative for CSF cytology in veterinary settings.

Urine microscopy

Urine analysis is one of the most widely used diagnostic tests in medicine, essential for the detection and monitoring of urinary tract infections (UTIs), kidney disease, metabolic disorders, and systemic conditions affecting renal function. Despite its routine use, urine microscopy remains a labor-intensive, variable, and partially subjective process, particularly when performed manually. Additionally, urine is a highly variable biological fluid providing an even greater challenge for manual operators and automated systems.

Proven in an independent clinical study[1], the compact, point-of-care analyzer that leverages DHM and AI-based object detection for automated, label-free urine sediment analysis, eliminated the need for centrifugation and staining, allowing for rapid near-patient testing.

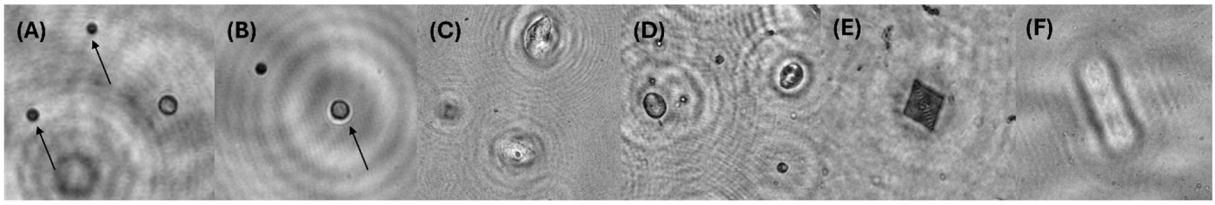

Figure 5. Holographic Images of (A) Red Blood Cells, (B) White Blood cell, (C) Squamous epithelial cell, (D) Non-squamous epithelial cells, (E) Crystal (F) Cast.

When benchmarked against traditional visual microscopy using Passing-Bablok regression and Spearman's correlation, the device shows distinct concordance for key clinical markers:

- **Red Blood Cells (RBCs):** Spearman's correlation coefficient of 0.86.
- **White Blood Cells (WBCs):** Spearman's correlation coefficient of 0.92.
- **Squamous Epithelial Cells (SECs):** Spearman's correlation coefficient of 0.94.

In this setting, the analyzer provides highly desirable imprecision profiles and meets linearity and Limits of Quantitation (LoQ) criteria as set by European Urinalysis Guidelines for RBCs and WBCs. It is a highly capable tool for rapid bedside screening of urinary tract and kidney diseases, though heavy reliance on finding specific crystal or cast pathologies might still require secondary verification via manual expert microscopy.

Cellular state and viability

Assessment of cellular state and viability is fundamental across biomedical research, bioprocessing and cell therapy manufacturing. Reliable determination of whether cells are viable, stressed, activated, apoptotic, or necrotic is critical for both clinical quality control and industrial applications. Current cellular viability technologies are dominated by biochemical staining and flow cytometry. DHM enables continuous, label-free monitoring of cell swelling, membrane dynamics, morphological changes and apoptosis progression, thus leveraging this information to obtain complex FACS-like results in an easy and fast one-step approach.

During viability measurement, each detected cell is analyzed by a trained algorithm that integrates morphological and optical markers into a continuous viability score from 0 (dead) to 1 (alive). This allows users to select the population of their interest but also have a deep and immediate insight into the health of their cell culture.

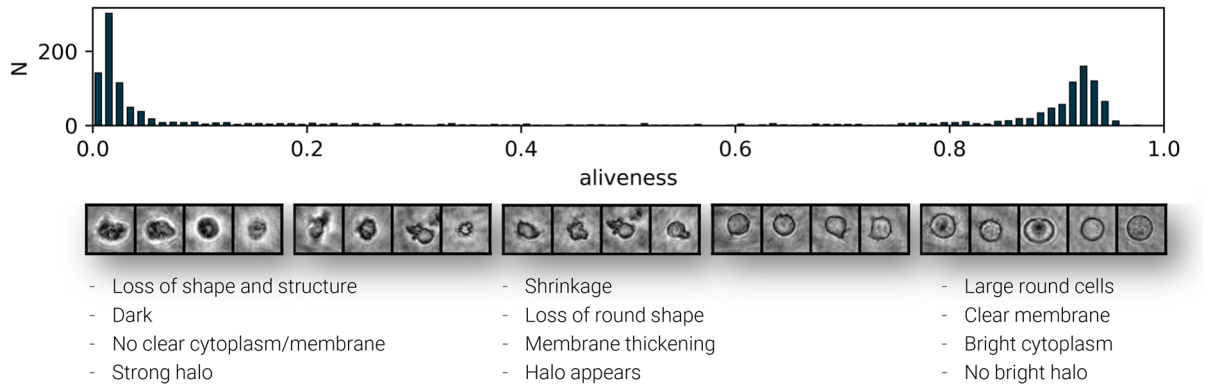

Figure 6. Different stages of cell death in DHM.

To evaluate the comparability of DHM, flow cytometry, and Trypan Blue staining for cell viability assessment, different experiments were conducted using both established cell lines and primary human cells. All experiments involved well-defined live-to-dead cell ratios, enabling direct comparison between methods underlying the remarkable performance of DHM in the detection of cellular viability. Further tested applications involve the impact of freeze, thaw cycles, UV irradiation, starvation and similar – proofing the feasibility of DHM for the detection of different types of cell death.

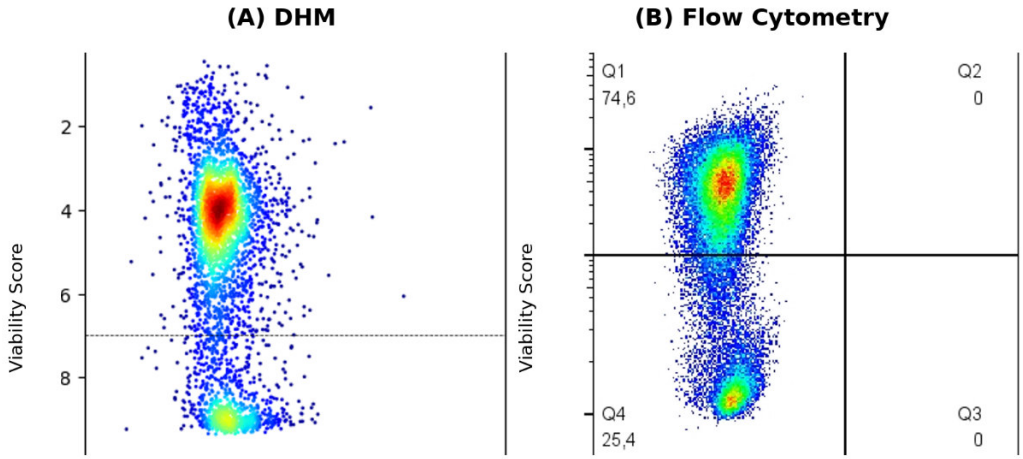

Figure 7 Comparison of viability plots generated from label-free DHM (A) and Flow cytometry after staining with Annexin (B). Jurkat cells treated with Staurosporin were spiked in healthy cells to obtain a target viability of 10% with Trypan blue.

Thus, holographic imaging introduced a powerful alternative to conventional methods for all these applications by enabling label-free, quantitative, and real-time analysis of living cells, with the potential to transform cell analytics across research, diagnostics, and biomanufacturing applications.

Conclusion

DHM represents a major evolution in clinical imaging and diagnostics. By combining label-free quantitative phase imaging with computational reconstruction and AI-assisted analysis, DHM addresses many limitations of conventional microscopy and flow cytometry systems. Thereby, its strengths include non-invasive imaging, minimal sample preparation and high potential for miniaturization and atomization, creating an optimized technology for integrative analyzer modules and standalone devices.

Future Perspectives

Future innovations in DHM currently under investigation include the integration of multiple light sources, nanoparticle-assisted contrast enhancement and high-resolution imaging. The use of engineered nanoparticles enables molecular targeting of specific cellular structures or biomarkers, significantly improving diagnostic specificity.

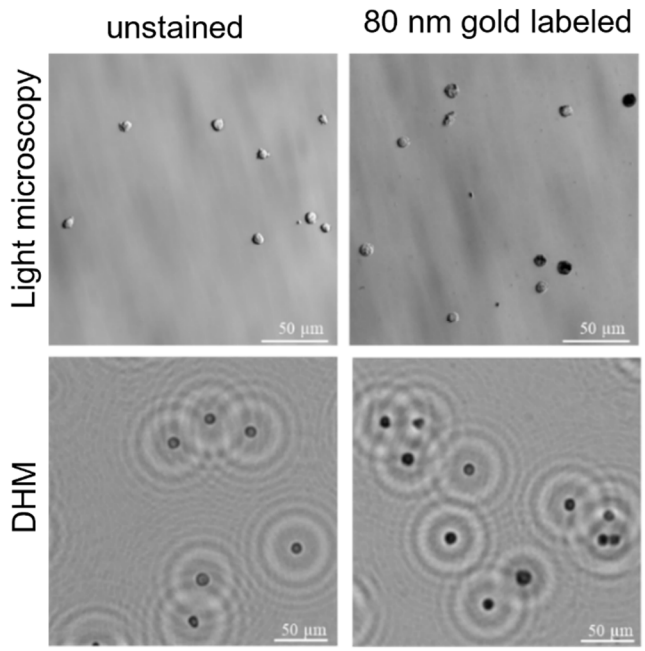

Figure 8.Changes in surface light intensity after antibody-specific labelling with 80 nm gold nanoparticle in Light Microscopy and DHM

At the same time, nanoparticle labeling enhances phase contrast signals, which is particularly beneficial for weakly scattering biological samples. The use of different particle types creates the potential for multiplex biomarker detection, allowing simultaneous analysis of multiple targets.

In parallel, advances in computational imaging increase the performance of DHM systems. Techniques such as pixel super-resolution overcome inherent sensor limitations, while multi-wavelength holography provides additional dimensions of information. In this context, multi-color imaging approaches enable simultaneous acquisition of complementary contrast channels while tomographic reconstruction methods enable accurate three-dimensional imaging of complex cellular structures.

Together, these approaches enable visualization at the subcellular level. As a result, DHM evolves into a high-resolution diagnostic platform that can compete with advanced optical microscopy techniques, while maintaining key advantages portability, robustness, and comparatively low system cost.

1) Deprez, Sigrid, Speeckaert, Marijn, Delanghe, Joris and Oyaert, Matthijs. "Analytical and diagnostic evaluation of the Anvajo fluidlab 2 analyzer: a novel urine particle analyzer for clinical application using digital holographic microscopy?" *Clinical Chemistry and Laboratory Medicine (CCLM)*, vol. 64, no. 5, 2026, pp. 1083-1092. https://doi.org/10.1515/cclm-2025-1202