Review Vol. 17, No. 5 / 1 May 2026 / Biomedical Optics Express 2349
Biomedical Optics EXPRESS

AI-driven digital holographic microscopy for label-free quantitative cellular analysis: toward low-cost and field-deployable platforms

INKYU MOON[1,*] AND BAHRAM JAVIDI[2]

[1]Department of Robotics & Mechatronics Engineering, DGIST, Daegu 42988, Republic of Korea
[2]Department of Electrical & Computer Engineering, University of Connecticut, Storrs, CT 06269, USA
*inkyu.moon@dgist.ac.kr

Abstract: Recent progress in artificial intelligence (AI) and digital holographic microscopy (DHM) has enabled quantitative, label-free, and noninvasive cellular imaging with unprecedented precision. This review provides an overview of AI-driven DHM technologies that transform classical holographic phase reconstruction and cellular analysis into real-time, portable biomedical solutions. After outlining the optical and computational fundamentals of DHM and quantitative phase imaging, we describe how deep generative and diffusion models substantially enhance phase retrieval accuracy under noisy or single-shot conditions. We then summarize recent biomedical applications, integrating blood, cancer, and cardiac cell analyses into a unified framework of AI-assisted quantitative phenotyping. Deep and self-supervised learning approaches are shown to enable high-accuracy classification of red blood cells and cancer cells and label-free evaluation of cardiomyocyte contractility and drug response. The combination of AI-based reconstruction, self-supervised learning, and physics-informed modeling demonstrates robust performance even with limited labeled data. Finally, we discuss the system-level transition toward low-cost, edge-AI-enabled DHM platforms capable of real-time phase imaging in point-of-care or field environments. We highlight key challenges in data standardization, interpretability, and multimodal integration. Collectively, this review envisions AI-integrated DHM as a scalable, accessible technology bridging advanced quantitative imaging with practical biomedical diagnostics.

1. Introduction

Quantitative assessment of biological cells at the single-cell level has been a long-standing objective in biomedical optics. Traditional optical microscopy techniques—such as bright-field, fluorescence, and confocal imaging—have played a central role in elucidating cell morphology and function. However, these approaches require labeling or staining that can perturb the intrinsic physiological state of living cells, introduce phototoxic effects, and limit long-term observation [1–4]. In recent years, label-free quantitative phase imaging (QPI) has emerged as a powerful alternative, capable of retrieving nanoscale optical path length variations without exogenous contrast agents. Among various QPI modalities, Digital Holographic Microscopy (DHM) has distinguished itself through its ability to capture the full optical field, including both amplitude and phase, in a single shot [5–8]. By recording the interference between an object and reference beam and numerically reconstructing the phase map through Fresnel diffraction, DHM provides quantitative information on cell morphology, refractive index, and dynamic motion at high temporal resolution [9–13]. The underlying physics of DHM enables real-time three-dimensional imaging of transparent biological samples.

In a typical off-axis configuration, a coherent reference beam is tilted slightly relative to the object beam, producing an interference pattern recorded as a hologram. After digital propagation, the object's complex amplitude can be reconstructed, yielding a quantitative phase map directly related to the specimen's optical thickness and refractive-index distribution [9,14]. This approach

#586407 https://doi.org/10.1364/BOE.586407
Journal © 2026 Received 1 Dec 2025; revised 22 Mar 2026; accepted 23 Mar 2026; published 13 Apr 2026

Biomedical Optics EXPRESS

has been successfully applied to red blood cell (RBC) analysis [7,15–19], tissue imaging [20,21], and cellular dynamics [22,23]. However, despite these advantages, classical DHM reconstruction faces several persistent limitations. Reconstruction accuracy depends on manual parameter tuning (e.g., propagation distance, filtering window), while the process is computationally intensive and sensitive to noise and twin-image artifacts. Moreover, the inverse problem of retrieving the complex object field from an intensity hologram is ill-posed under partially coherent illumination or system misalignment.

To overcome these challenges, recent research has increasingly incorporated Artificial Intelligence (AI) and data-driven methods into the DHM pipeline [8,24–31]. Deep convolutional neural networks (CNNs) [32,33], generative adversarial networks (GANs) [34], and, more recently, diffusion and self-supervised models [35–37] have redefined the landscape of phase recovery and analysis [38–43]. Moon et al. [8] demonstrated a conditional GAN (cGAN) architecture that reconstructs noise-free quantitative phase images from single in-line Gabor holograms, effectively suppressing twin-image interference. Following this, Park et al. [31] introduced a diffusion-based unsupervised model capable of high-fidelity single-shot phase retrieval without paired training data. These approaches leverage large datasets of holograms to learn statistical priors of natural holographic structures, replacing explicit physical modeling with learned implicit propagation. Such learning-based reconstruction not only accelerates processing but also increases robustness to experimental noise, enabling real-time quantitative phase imaging on portable hardware.

Beyond image reconstruction, AI has also transformed cellular classification and functional analysis in DHM. In the hematology field, deep learning models have been applied to automatically assess RBC storage lesions, which represent morphological and biochemical degradations during blood preservation [7,26,44]. Kim et al. [26] combined GAN-based image enhancement with watershed segmentation to perform phenotypic classification of stored RBCs, achieving high accuracy in differentiating discocytes, echinocytes, and spherocytes. Similarly, Jaferzadeh et al. [7] used time-lapse holography to quantify membrane fluctuations as a measure of deformability loss. More recent work by Moon et al. [30] proposed a self-supervised diffusion framework integrating compact holographic sensors, achieving real-time RBC quality assessment on embedded devices. Together, these advances position AI-assisted DHM as a promising noninvasive tool for transfusion safety and automated blood-bank quality control.

In cancer research, DHM combined with machine learning has enabled stain-free phenotypic classification of malignant cells. Jaferzadeh et al. [45] systematically compared handcrafted morphological descriptors and CNN-based deep features for elliptical cancer cell identification, reporting approximately 9% higher accuracy with the deep learning model. Complementary to this, Rehman et al. [29] applied self-supervised learning frameworks—SimCLR [46], Barlow Twins [47], and SwAV [48] —to off-axis holographic datasets, demonstrating that robust classification can be achieved even when only 20% of the data are labeled. Such strategies are particularly valuable in biomedical imaging, where annotated datasets are limited or difficult to obtain. By learning latent representations directly from unlabeled holograms, self-supervised models extend the scalability of DHM to a wider range of clinical and biological studies.

Functional imaging of living cardiac cells provides another example of the versatility of AI-driven DHM. Moon et al. [49] quantified three-dimensional beating profiles of hiPSC-derived cardiomyocytes at the single-cell level using DHM, while a subsequent study [50] combined time-lapse holography with transfer learning to assess drug-induced cardiotoxicity. Unlike fluorescence-based calcium imaging, this approach is entirely label-free, reducing phototoxic stress and enabling continuous monitoring over extended periods. When paired with deep learning for spatiotemporal pattern recognition, DHM offers a pathway to high-content screening and predictive toxicology without molecular labeling. In parallel with algorithmic progress, system-level innovations have targeted hardware miniaturization and field deployability. Recent work [30] demonstrated an integrated DHM sensor performing deep inference directly on

Biomedical Optics EXPRESS

embedded GPUs, achieving over 30 frames per second for holographic reconstruction and classification. Such compact configurations, when combined with edge-AI processing, pave the way toward point-of-care diagnostics, telemedicine, and environmental monitoring. They also align with the broader vision of low-cost, high-throughput digital biophotonic sensors capable of operating outside traditional laboratory environments.

This review paper aims to consolidate these multidisciplinary developments by presenting a unified overview of AI-driven DHM for label-free quantitative cellular analysis. The overall workflow of AI-driven DHM—from hologram acquisition to AI-assisted phase reconstruction and subsequent quantitative cellular phenotyping—is summarized in Fig. 1. Section 2 revisits the physical and computational fundamentals of DHM, from interference recording to phase reconstruction. Section 3 focuses on AI-enhanced phase recovery and holographic reconstruction methods, including supervised, hybrid physics-guided, generative, and diffusion-based learning frameworks. Section 4 integrates the diverse biomedical applications—spanning blood, cancer, and cardiac cell imaging—into a cohesive framework of AI-assisted quantitative phenotyping and cellular dynamics analysis. Section 5 discusses system-level advances toward portable, field-deployable DHM platforms, including real-time and edge-AI-enabled holographic sensing architectures. Section 6 highlights challenges in data standardization, explainability, and multimodal integration. Finally, Section 7 concludes with perspectives on how AI-integrated DHM can transition from a laboratory technique to a globally accessible diagnostic platform. By bridging optical physics and modern machine intelligence, this review envisions DHM as a transformative technology for the next generation of quantitative, label-free biomedical imaging.

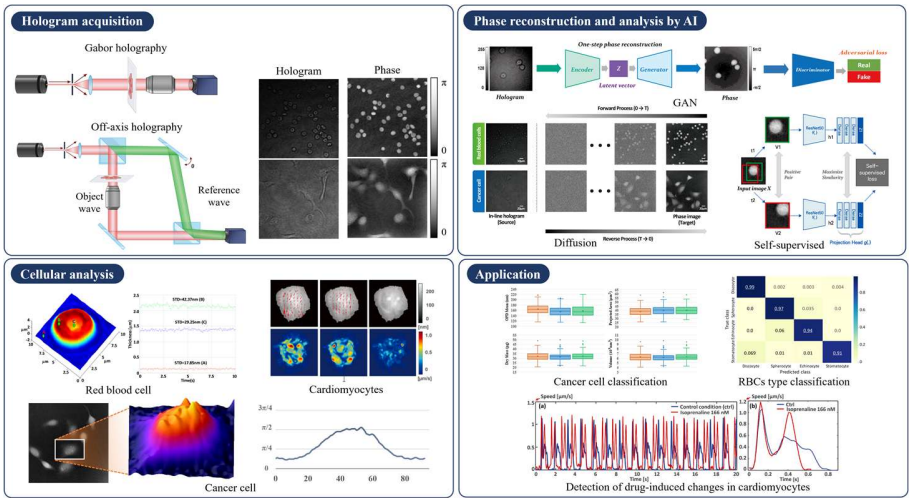

Fig. 1. Schematic overview of the AI-driven DHM pipeline. Raw holograms are recorded through optical interference between object and reference beams and reconstructed into quantitative phase maps using AI-assisted reconstruction models (supervised, generative, and diffusion-based networks). The reconstructed phase images are then analyzed for cellular phenotyping of biological samples such as red blood cells, cancer cells, and cardiomyocytes.

2. Optical and computational principles of DHM

Digital holographic microscopy (DHM) is a coherent imaging technique that retrieves both amplitude and phase information from the interference between a reference and an object beam. The basic principle is straightforward: a coherent light field transmitted or reflected by a specimen, $O(x,y)$, interferes with a reference beam $R(x,y)$, and the resulting intensity hologram is recorded

Biomedical Optics EXPRESS

on a camera sensor. The recorded pattern can be expressed as

$$I_H(x,y) = |O(x,y) + R(x,y)|^2 = |O|^2 + |R|^2 + R^*O + O^*R, \tag{1}$$

where the cross-term R^*O carries the object's amplitude and phase information. In off-axis DHM, the reference beam is slightly tilted, spatially separating this term in the Fourier domain and allowing direct reconstruction of the complex wavefront [9]. Alternatively, in-line (Gabor) holography offers a simpler optical design by using a common-path geometry but produces overlapping orders that require computational or AI-based suppression [8].

To recover the complex object field from the recorded intensity, numerical propagation based on Fresnel diffraction is employed. A practical implementation uses a Fourier-domain approach:

$$U(x,y;z) = F^{-1}\{F[I_H(x,y)]H(f_x,f_y;z)\}, \tag{2}$$

where $H(f_x,f_y;z) = \exp[ikz]\exp[-i\pi\lambda z(f_x^2+f_y^2)]$ is the Fresnel transfer function, $k = 2\pi/\lambda$ is the wavenumber, and λ is the illumination wavelength. This compact form emphasizes that numerical reconstruction is essentially a filtering operation in the spatial-frequency domain.

After inverse transformation, the resulting complex amplitude $U(x,y;z)$ contains both intensity and phase, which can be refocused digitally by varying z. The recovered phase $\phi(x,y)$ represents the optical path difference (OPD) introduced by the sample:

$$OPD(x,y) = h(x,y)[n_c(x,y) - n_m], \tag{3}$$

$$\phi(x,y) = \frac{2\pi}{\lambda} OPD(x,y), \tag{4}$$

where $h(x,y)$ denotes the local sample thickness, n_c the refractive index of the cell, and n_m that of the surrounding medium. These equations provide the physical basis of quantitative phase imaging (QPI), establishing a direct link between optical phase and biophysical properties such as cell morphology, dry-mass density, and refractive index contrast [7,8,12].

DHM has been implemented in both transmission and reflection geometries. In biological applications, the transmission mode is more common because most cells are optically transparent and require high phase sensitivity rather than intensity contrast. High-resolution off-axis systems have been applied to red blood cells, neurons, and cardiomyocytes, whereas compact in-line systems support portable or on-chip implementations [30,31]. The combination of digital reconstruction and phase analysis enables label-free, quantitative visualization of cellular dynamics—forming the foundation upon which AI-driven enhancement and classification methods are built, as discussed in Section 3.

3. AI-enhanced phase recovery and image reconstruction

Digital holographic microscopy (DHM) enables label-free quantitative phase imaging by computationally reconstructing phase information from recorded holograms. In recent years, artificial intelligence has emerged as a powerful computational tool for improving the robustness and quality of holographic reconstruction, particularly under noisy, defocused, or photon-limited imaging conditions. In this section, we focus specifically on AI approaches that directly participate in the recovery or reconstruction of phase information from holographic measurements. These methods aim to enhance or replace conventional numerical reconstruction by learning statistical priors of holographic data and phase structure. Throughout this section, the role of AI is therefore restricted to the phase-recovery stage of the holographic pipeline, including direct hologram-to-phase reconstruction, physics-guided phase retrieval, and generative reconstruction approaches. To avoid conceptual ambiguity, studies in which AI is applied only after phase reconstruction—for example for cellular segmentation, classification, or phenotyping using

Biomedical Optics EXPRESS

already reconstructed phase images—are discussed separately in Section 4. This distinction clarifies the different computational roles of AI within the DHM workflow and ensures that the methods reviewed in this section address a consistent task: AI-assisted holographic phase recovery and image reconstruction.

3.1. Background: from physics-based to learning-based reconstruction

Digital holographic microscopy (DHM) traditionally relies on numerical propagation algorithms based on the Fresnel diffraction integral [8]. As described in Section 2, the complex field $U(x,y;z)$ is reconstructed by propagating the recorded hologram $I_H(x,y)$ through the Fresnel kernel, followed by phase unwrapping [51–53] and aberration correction [54]. While this framework has enabled the quantitative measurement of cellular morphology and refractive index, it is inherently sensitive to defocus, speckle noise, and system aberrations. Manual selection of the reconstruction distance z and filtering parameters often leads to inconsistent results between experiments. Moreover, under in-line (Gabor) configurations [5,55], twin-image artifacts—the out-of-focus conjugate of the object wave—introduce severe degradation of phase accuracy. Although iterative phase retrieval algorithms such as Gerchberg–Saxton or transport-of-intensity methods can suppress these artifacts, they are computationally slow and prone to local minima [56,57].

The advent of deep learning (DL) has offered a transformative alternative to these physics-based numerical reconstructions. Rather than explicitly modeling diffraction, AI models learn the inverse mapping from raw holograms to clean quantitative phase maps directly from data. This paradigm shift—from deterministic propagation to data-driven inference—has led to a new generation of AI-driven DHM, capable of fast, noise-resilient, and often single-shot reconstruction [8,26,29–31]. AI models increasingly function as data-driven surrogates for physical propagation, learning statistical priors of diffraction and phase evolution from large holographic datasets.

3.2. Deep generative models for noise-free phase imaging

The first major breakthrough in AI-based phase reconstruction came from the adoption of Generative Adversarial Networks (GANs) [34]. A GAN consists of two networks trained in opposition—a generator G that synthesizes images and a discriminator D that distinguishes real from synthetic outputs. The training objective follows the classical min–max loss function:

$$L_{GAN} = E_{x\sim p_{data}(x)}[\log D(x)] + E_{z\sim p_z(z)}[\log(1 - D(G(z)))], \tag{5}$$

where x denotes real phase data and z represents a random latent variable. The generator learns to approximate the mapping from a noisy hologram to a realistic, artifact-free phase map.

Moon et al. [8] were among the first to apply a conditional GAN (cGAN) to holography. Their model reconstructed quantitative phase images directly from single in-line Gabor holograms, removing twin-image noise and improving the signal-to-noise ratio (SNR) by up to 12 dB compared with classical Fresnel propagation. The cGAN architecture used paired hologram–phase datasets for supervised training and generated reconstructions that preserved fine cellular morphology without requiring iterative optimization. This approach demonstrated that deep generative priors can effectively substitute for explicit phase-retrieval constraints.

Following this work, several studies extended the GAN framework for different reconstruction objectives—such as autofocusing, speckle suppression, and phase unwrapping. For example, U-Net and encoder–decoder architectures have been trained to predict the best focal plane from a defocused hologram sequence, dramatically reducing computational time [8,30,31]. These models act as learned propagators, embedding both optical and statistical knowledge of diffraction into the network weights. The principle of GAN-based phase recovery and its typical architecture are depicted in Fig. 2.

Biomedical Optics EXPRESS

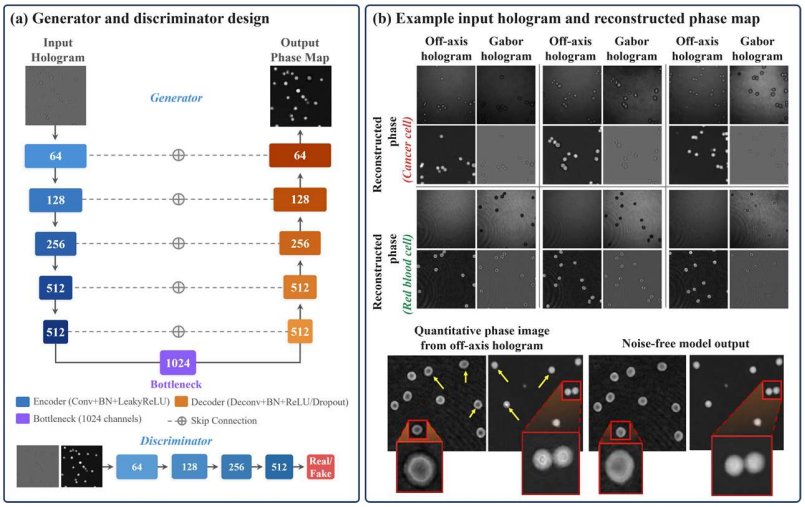

Fig. 2. Architecture of the conditional generative adversarial network (cGAN) for phase reconstruction [8]. (a) Generator and discriminator design; (b) example input hologram and reconstructed phase map demonstrating effective twin-image suppression.

3.3. *Diffusion-based unsupervised holographic reconstruction*

While GANs require paired training datasets, acquiring ground-truth phase images is labor-intensive and often impractical for biological samples. To address this, Park et al. [31] proposed an unsupervised diffusion model for single-shot holographic phase recovery. Diffusion models, originally developed for generative image synthesis, learn to gradually denoise a sample by reversing a known noise-adding process [35,36]. The forward process can be represented as

$$q(x_t|x_{t-1}) = N\left(x_t; \sqrt{1-\beta_t}x_{t-1}, \beta_t I\right), \tag{6}$$

where β_t is the variance schedule controlling the amount of Gaussian noise injected at each timestep t. During training, the model learns to predict and remove this noise, effectively reconstructing the clean phase x_0 from its corrupted versions. In the context of DHM, this probabilistic framework has two major advantages. First, it does not require explicit phase labels, making it data-efficient and applicable to raw holograms collected under diverse conditions. Second, diffusion models yield high-fidelity reconstructions with smooth global phase continuity, outperforming conventional supervised CNNs and even GANs in terms of quantitative accuracy. In experiments with biological holograms of RBCs and fibroblast cells, the unsupervised diffusion model achieved phase error reductions of over 25% relative to cGANs [31]. The model's stochastic sampling also provides a measure of reconstruction uncertainty, an important step toward interpretable AI in biomedical optics. The diffusion-based unsupervised reconstruction process, outlined in Fig. 3, provides smooth, noise-free phase maps using probabilistic denoising.

3.4. *Supervised & hybrid deep-learning approaches for quantitative phase reconstruction*

In addition to generative and diffusion-based approaches, supervised and hybrid deep-learning frameworks have been widely explored for quantitative phase reconstruction in digital holography. These approaches typically rely on paired training datasets or integrate physical priors into neural networks in order to improve reconstruction accuracy and suppress imaging artifacts. A representative example is the deep-learning-based holographic reconstruction method proposed

Biomedical Optics EXPRESS

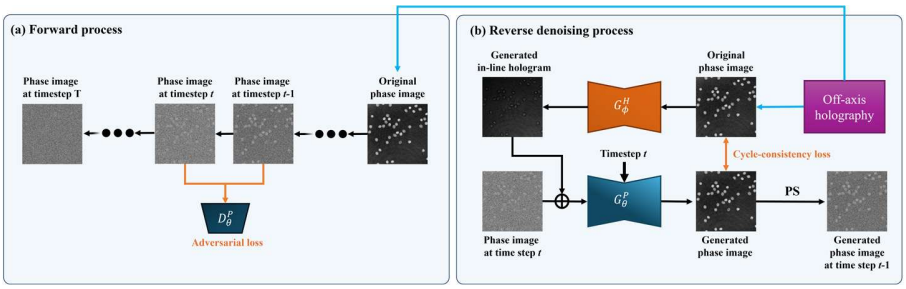

Fig. 3. Concept of diffusion-based holographic reconstruction [31]. (a) Forward process gradually adds Gaussian noise to holograms; (b) reverse denoising process recovers the clean phase. The model achieves high fidelity without paired training data.

by Di et al., which introduced a convolutional neural network called PhaseNet for quantitative phase imaging using digital holographic microscopy [58]. In this framework, the network directly reconstructs phase information from recorded holograms, effectively replacing conventional numerical propagation and phase-unwrapping procedures. Experimental results demonstrated accurate recovery of phase maps from biological samples while preserving fine cellular structural details.

Hybrid learning strategies that combine physical models with neural networks have also been proposed to enhance reconstruction robustness. Li et al. developed a deep-learning-assisted variational Hilbert quantitative phase imaging (DL-VHQPI) framework that integrates a variational Hilbert phase-demodulation model with a neural network for residual error compensation [59]. In this approach, the analytical model extracts fringe information and provides strong physical constraints, while the neural network refines the reconstruction by recovering spectral components that cannot be captured by the analytical model. This hybrid strategy significantly reduces the amount of required training data while maintaining high reconstruction accuracy. Another important direction is end-to-end deep-learning reconstruction, in which neural networks directly learn the mapping from raw holograms to reconstructed images. Ren et al. proposed the holographic reconstruction network (HRNet), which performs holographic reconstruction in a fully data-driven manner [40]. Unlike traditional numerical propagation methods that require prior knowledge of system parameters, the network automatically learns the reconstruction process and suppresses artifacts such as twin images and phase aberrations.

Deep learning has also been applied to quantitative phase imaging of biological cells using digital holographic microscopy. Nguyen et al. demonstrated a deep-learning-based framework (VY-Net) for quantitative phase imaging of living red blood cells from single-shot off-axis digital holograms [60]. In this approach, the neural network reconstructs the phase image directly from the recorded hologram, enabling accurate recovery of cellular morphology and quantitative biophysical parameters without conventional phase-demodulation procedures. More recently, deep learning has been employed to enhance spatial resolution in holographic reconstruction. Park et al. proposed a deep-learning framework that simultaneously performs phase reconstruction and super-resolution from low-resolution holograms [28]. By integrating image translation and super-resolution modules, the model generates high-resolution phase images while preserving fine structural details of biological samples. Collectively, these studies illustrate several key paradigms in AI-assisted holographic reconstruction, including supervised CNN-based phase reconstruction, physics-guided hybrid learning, end-to-end hologram-to-image networks, biomedical quantitative phase imaging of living cells, and deep-learning-based resolution enhancement. These approaches complement generative and diffusion-based models and further expand the capabilities of quantitative phase imaging in digital holographic microscopy.

Biomedical Optics EXPRESS

3.5. Comparative assessment and future trends

Recent advances in AI-assisted holographic phase reconstruction demonstrate a clear evolution from traditional physics-based reconstruction toward learning-driven computational imaging frameworks. Classical digital holographic reconstruction methods rely on analytical diffraction models such as Fresnel propagation and angular spectrum methods (ASMs), which provide physically interpretable phase recovery but often require careful parameter tuning and iterative optimization. These limitations have motivated the development of deep-learning-based approaches that can directly learn mappings between holographic measurements and quantitative phase images. Early learning-based reconstruction studies primarily employed supervised convolutional neural networks to perform direct hologram-to-phase reconstruction. Architectures such as U-Net and PhaseNet demonstrated that neural networks can significantly accelerate phase recovery while suppressing artifacts such as twin images and phase noise. These supervised CNN-based frameworks represent one of the earliest demonstrations that deep learning can effectively replace conventional numerical propagation in digital holographic microscopy.

Subsequent studies explored generative learning frameworks to further improve reconstruction quality. Conditional generative adversarial networks (GANs) were introduced to enhance image realism and improve the signal-to-noise characteristics of reconstructed phase images by learning statistical priors of biological structures. More recently, diffusion-based generative models have emerged as powerful tools for holographic reconstruction. Diffusion models learn the distribution of artifact-free phase images and iteratively refine noisy holographic reconstructions through stochastic denoising processes, providing improved robustness to noise and experimental variability while reducing the dependence on paired training datasets. In addition to these generative reconstruction paradigms, hybrid deep-learning strategies have been proposed to combine physical interpretability with data-driven modeling. For example, variational Hilbert-based quantitative phase imaging integrates analytical fringe-demodulation models with neural networks to compensate residual reconstruction errors. This hybrid approach preserves the physical structure of the reconstruction model while benefiting from the error-correction capability of deep learning.

Recent studies have further extended AI-assisted holographic reconstruction to practical biomedical imaging applications. Deep-learning-based frameworks have demonstrated quantitative phase reconstruction of living red blood cells directly from digital holograms, enabling accurate recovery of cellular morphology and biophysical parameters in label-free measurements. In addition, deep learning has been employed to enhance spatial resolution in holographic imaging. Super-resolution frameworks integrate phase reconstruction and resolution enhancement within a unified neural architecture, allowing high-resolution phase images to be generated from low-resolution holographic measurements. Table 1 summarizes representative reconstruction approaches discussed in Sections 3.1–3.4 and compares their imaging geometries, learning paradigms, data requirements, and reconstruction characteristics. As shown in the table, supervised CNN-based reconstruction, generative adversarial learning, diffusion-based models, hybrid physics-learning frameworks, and deep-learning-based super-resolution collectively illustrate the rapid evolution of AI-assisted holographic phase reconstruction.

Overall, these developments have transformed digital holographic microscopy from a computationally intensive imaging technique into a scalable and automated quantitative imaging platform. The integration of physics-based models and data-driven learning frameworks is expected to play a central role in future holographic imaging systems, enabling robust quantitative phase imaging for biomedical diagnostics, cell analysis, and real-time computational microscopy.

Biomedical Optics EXPRESS

Table 1. Comparison of representative AI architectures used for holographic phase reconstruction

Method / Architecture	Imaging Geometry	Learning Mode	Ground-Truth Requirement	AI Role in Pipeline	Key Contribution	Processing Speed
Physics-Based Reconstruction (Fresnel / ASM) [9]	In-line / Off-axis	Analytical	None	Numerical phase retrieval	Baseline numerical reconstruction using wave propagation models	Moderate
CNN / U-Net Reconstruction [8]	In-line / Off-axis	Supervised	Paired	Direct phase reconstruction	Direct mapping from hologram intensity to quantitative phase	High
Conditional GAN Reconstruction [8]	In-line	Supervised generative	Paired	Reconstruction refinement	Improved phase realism and noise suppression	High
Diffusion-Based Holographic Reconstruction [31]	In-line	Generative diffusion	None / minimal	Iterative phase reconstruction	High-fidelity phase recovery through probabilistic denoising	Moderate
Deep Learning-Based Holographic Reconstruction (PhaseNet) [58]	In-line	Supervised	Paired	Direct phase reconstruction	CNN-based quantitative phase imaging from single hologram	High
DL-Assisted Variational Hilbert QPI [59]	In-line	Hybrid (Physics + DL)	Minimal	Physics-guided phase retrieval	Artifact-reduced phase reconstruction combining Hilbert transform and DL	Moderate
End-to-End Deep Learning Reconstruction (HRNet) [40]	In-line	Supervised	Paired	Direct hologram-to-phase reconstruction	End-to-end deep network replacing numerical propagation	High
Deep Learning for RBC QPI (VY-Net) [60]	Off-axis	Supervised	Paired	Direct phase reconstruction	Single-shot phase reconstruction of living RBCs in DHM	High
Deep Learning Phase Reconstruction with Super-Resolution [28]	In-line	Supervised	Paired / synthetic	Reconstruction + resolution enhancement	Simultaneous phase recovery and spatial resolution improvement	Moderate

4. Applications in biomedical cell imaging and functional assessment

4.1. Real-time AI-enabled holographic sensing platforms

Recent advances in AI-enhanced holographic reconstruction, discussed in Section 3, have significantly improved the accuracy and robustness of quantitative phase recovery. Beyond

Biomedical Optics EXPRESS

algorithmic performance, however, an important emerging direction is the deployment of these reconstruction methods in real-time sensing systems for practical biomedical applications. Real-time applications—such as point-of-care diagnostics—require compact and computationally efficient models that can operate directly within imaging hardware. Moon et al. [30] introduced a holographic sensor integrated with deep learning that performs on-device inference using an embedded GPU. By combining model compression, pruning, and quantization, they achieved inference speeds exceeding 30 frames s^{-1} for hologram reconstruction and RBC classification. This work demonstrated that AI-enhanced DHM can be implemented not only as a post-processing step but also as a fully integrated sensing pipeline. The approach enables edge-AI holography, where phase retrieval, denoising, and feature extraction occur directly on a compact module without external computation. Such efficiency improvements align with broader trends in biomedical imaging toward portable, cloud-connected systems.

Real-time holographic reconstruction using lightweight CNNs or diffusion models allows continuous monitoring of dynamic biological processes, such as cardiomyocyte beating or RBC membrane fluctuation. Furthermore, the combination of hardware miniaturization and neural optimization enables the deployment of AI-DHM in resource-limited environments, from blood-bank quality control to telemedicine and environmental biosensing [29,30,45]. Figure 4 shows an example of the edge-AI holographic sensor capable of on-device phase retrieval and cellular assessment.

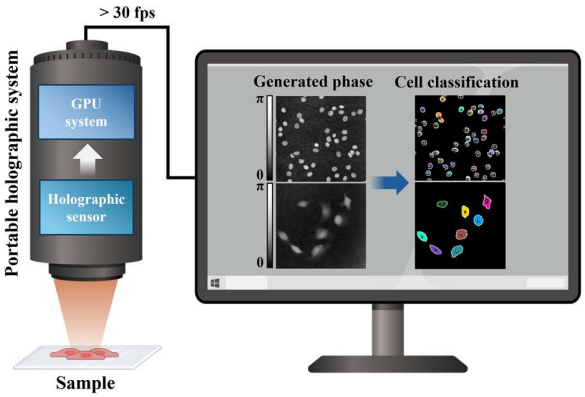

Fig. 4. Portable holographic sensor integrated with deep learning inference [30]. The embedded GPU executes reconstruction and classification in real time within a compact optical module.

4.2. *Quantitative analysis of blood cells*

Red blood cells (RBCs) are an ideal model for evaluating the capability of DHM in label-free biophysical analysis because they are optically transparent and exhibit well-defined morphological transformations during storage and disease progression. Early DHM studies quantified membrane fluctuations and morphological indices such as sphericity, surface-to-volume ratio, and dry mass, providing insight into cell deformability and viability [7,44]. Jaferzadeh et al. [7] used time-lapse DHM to measure nanometer-scale fluctuations of stored RBC membranes, revealing a progressive decrease in fluctuation amplitude that correlated with reduced deformability. Yi et al. [44] further implemented statistical classifiers based on Gabor-filter features extracted from holographic phase maps to distinguish RBC morphological categories such as discocytes, echinocytes, and spherocytes. These early methods established the feasibility of quantitative, label-free RBC phenotyping using DHM but still relied on manual feature extraction and classical machine-learning pipelines.

Biomedical Optics EXPRESS

The introduction of AI-based RBC analysis dramatically improved automation and accuracy. Kim et al. [26] employed deep learning for automated phenotypic assessment of RBC storage lesions, integrating phase maps with a generative adversarial network (GAN)-assisted image enhancement stage and a watershed segmentation module. This framework achieved above 90% classification accuracy and provided direct correlation between morphological phenotype and storage duration. Building on this concept, Moon et al. [30] integrated a holographic sensor with self-supervised diffusion learning for real-time, noninvasive RBC quality assessment. Their edge-AI system inferred morphological quality indices directly on a compact embedded GPU, enabling on-site blood-bank monitoring without chemical reagents or labeling. Together, these works demonstrate that AI-integrated DHM can bridge the gap between biophysical measurements and practical transfusion diagnostics. From a quantitative standpoint, DHM provides the total dry mass M of a single RBC by integrating the optical phase map:

$$M = \frac{\lambda}{2\pi\alpha} \iint \phi(x, y) dx dy, \tag{7}$$

where λ is the illumination wavelength and $\alpha \approx 0.18$ mL/g is the commonly used refractive index increment for hemoglobin. This parameter directly reflects hemoglobin content and has been used to evaluate oxygen-carrying capacity and pathological conditions such as anemia or hemoglobinopathies [7]. By coupling Eq. (7) with AI-based feature extraction, recent systems can automatically generate statistical distributions of cellular mass and sphericity across thousands of holographically imaged cells in seconds.

Recent developments have further extended AI-based RBC analysis through self-supervised and physics-informed learning strategies. Self-supervised learning leverages unlabeled holographic datasets by defining auxiliary representation-learning tasks that do not require explicit phase annotations. For example, representation learning frameworks such as contrastive learning can exploit variations in defocus or holographic contrast to learn invariant features of RBC morphology, reducing dependence on large labeled datasets. Such approaches are particularly beneficial in biomedical DHM applications where obtaining reliable ground-truth phase annotations can be challenging. In parallel, physics-informed learning approaches incorporate optical propagation models directly into the training process. By embedding diffraction operators or transport-of-intensity constraints into neural network optimization, these models enforce consistency with known physical laws while enabling data-driven correction of noise and aberrations. This hybrid framework preserves the interpretability of physics-based reconstruction while improving robustness in practical experimental conditions. Figure 5 illustrates how DHM combined with AI enables quantitative, label-free monitoring of RBC morphology during storage.

4.3. Cancer cell phenotyping and classification

Cancer cells exhibit subtle morphological differences that are difficult to capture using conventional bright-field imaging, especially when unstained. The label-free quantitative phase contrast provided by DHM offers a unique opportunity for automated cancer phenotyping, and AI has proven essential for reliable classification. Jaferzadeh et al. [45] developed a stain-free DHM framework for the phenotypic classification of elliptical cancer cells derived from lung, breast, and skin tissues. By comparing traditional feature-based machine learning (support-vector machines, random forests, and artificial neural networks) with deep convolutional models (ResNet-50), they showed that deep networks achieved approximately 95% accuracy—roughly 9% higher than handcrafted features. The phase-retrieved images captured with DHM provided morphological cues related to nuclear irregularity and cytoplasmic granularity that are invisible to conventional microscopy.

Expanding on this direction, Rehman et al. [29] employed self-supervised learning (SSL) methods such as SimCLR, Barlow Twins, and SwAV to handle large unlabeled holographic

Biomedical Optics EXPRESS

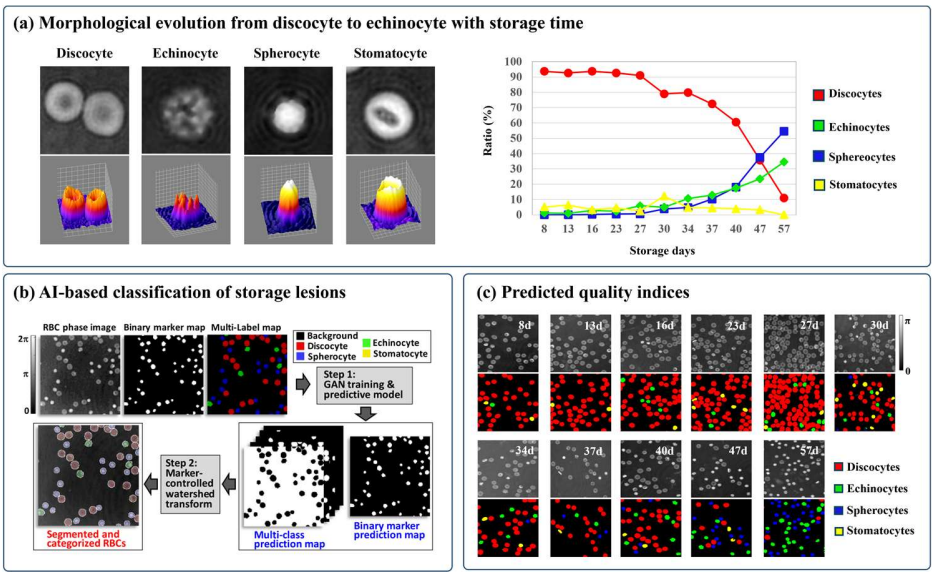

Fig. 5. Representative DHM phase images of stored RBCs [26]. (a) Morphological evolution from discocyte to echinocyte with storage time; (b) AI-based classification of storage lesions; (c) predicted quality indices from self-supervised diffusion learning.

datasets of elliptical cancer cells. These SSL models achieved comparable performance to supervised networks even when trained with only 10–20% labeled data, highlighting the potential of AI-DHM for scalable cytological screening in limited-annotation settings. By learning invariant representations of phase patterns across defocus and illumination variations, SSL approaches improve generalization across different imaging systems—an essential feature for clinical deployment. The combination of deep and self-supervised learning thus allows DHM to function as a label-free digital cytometer, capable of classifying diverse cell populations without staining. Beyond classification, ongoing research explores the extraction of refractive-index gradients, dry-mass distributions, and nanoscale texture descriptors from DHM phase maps for predicting metastatic potential and drug response. Such quantitative biophysical biomarkers could complement molecular assays in personalized oncology. Deep and self-supervised learning frameworks for label-free cancer cell classification are compared in Fig. 6.

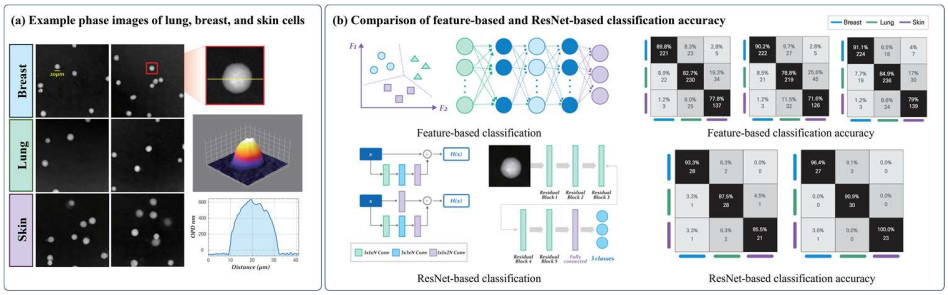

Fig. 6. Elliptical cancer cell classification using DHM [45]. (a) Example phase images of lung, breast, and skin cells; (b) comparison of feature-based and ResNet-based classification accuracy (~95%).

Biomedical Optics EXPRESS

4.4. Functional imaging of cardiomyocytes

The capability of DHM to measure dynamic phase changes with nanometer precision makes it well suited for functional imaging of beating cardiac cells. Moon et al. [49] used three-dimensional DHM to record contractile motion of hiPSC-derived cardiomyocytes at single-cell resolution. The time-resolved optical path difference (OPD) fluctuations corresponded directly to mechanical contraction and relaxation, enabling noninvasive quantification of beating amplitude and frequency. Because DHM requires neither fluorescent dyes nor mechanical contact, it minimizes phototoxicity and allows long-term observation of cardiomyocyte physiology.

Subsequently, Moon et al. [50] combined DHM with transfer learning to analyze drug-treated cardiomyocytes and automatically detect cardiotoxicity. By training deep networks on temporal phase sequences, they identified characteristic changes in beating periodicity and propagation speed induced by known cardiotoxic compounds. This label-free, high-content approach offers an alternative to fluorescence-based calcium imaging for evaluating drug safety. The integration of AI and DHM enables high-throughput analysis of thousands of cells simultaneously, capturing both morphological and functional endpoints in a single imaging modality. Beyond drug screening, AI-enhanced DHM provides opportunities for personalized medicine. By quantifying patient-specific cardiomyocyte contractility and predicting functional abnormalities, DHM could support individualized therapy design for cardiac diseases. The compactness of modern holographic systems [30] further suggests future point-of-care implementations where on-chip DHM modules perform continuous monitoring of engineered cardiac tissues or organ-on-chip systems. The capability of DHM to record cardiomyocyte beating and drug response is visualized in Fig. 7.

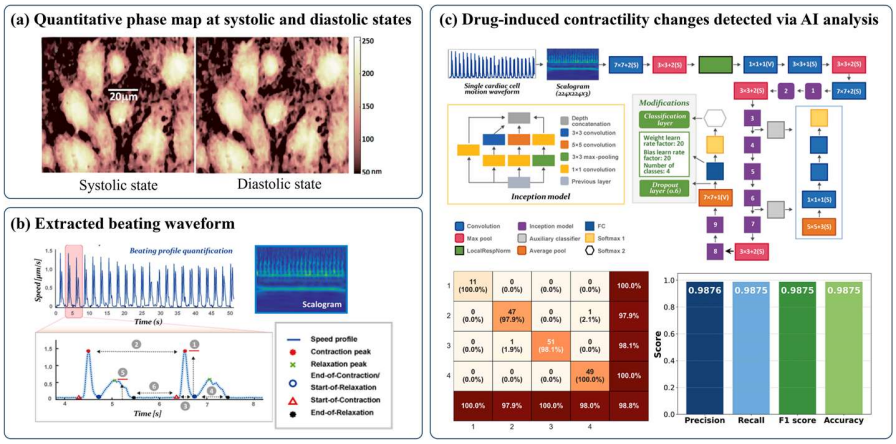

Fig. 7. Time-resolved DHM of beating cardiomyocytes [49,50]. (a) Quantitative phase map at systolic and diastolic states; (b) extracted beating waveform; (c) drug-induced contractility changes detected via AI analysis.

4.5. Deep-learning–based cellular analysis and disease classification in DHM

Beyond specific applications such as red blood cell characterization, cancer cell phenotyping, and cardiomyocyte functional imaging, recent studies have demonstrated that deep learning can enable generalized cellular analysis directly from digital holographic microscopy (DHM) data. These approaches extend AI-assisted holography from simple phase reconstruction toward automated interpretation of cellular morphology and dynamics.

One representative direction involves automated detection and counting of microscopic phase objects directly from raw holograms. A deep-learning framework was proposed to identify

Biomedical Optics EXPRESS

and enumerate phase objects without explicit holographic reconstruction [61]. By training convolutional neural networks on holographic intensity patterns, the model can infer object locations and counts directly from raw DHM measurements, thereby bypassing computationally expensive reconstruction steps and enabling rapid analysis of dense cellular populations. Another important line of work focuses on segmentation of individual cells in quantitative phase images. The DL-CSPF framework introduced a deep-learning–based cell segmentation method that incorporates a physical imaging model into the learning architecture [62]. By embedding optical constraints into the neural network training process, this hybrid approach improves segmentation accuracy while maintaining consistency with the underlying holographic imaging physics.

Deep learning has also enabled functional characterization of cellular states using quantitative phase dynamics. For example, DHM combined with neural networks has been used to discriminate between apoptosis and necroptosis, two forms of programmed cell death that exhibit subtle morphological differences [63]. By analyzing temporal phase variations in living cells, the model can identify early-stage cellular responses that are difficult to distinguish using conventional imaging techniques. More recently, compact DHM systems integrated with deep learning have demonstrated the ability to identify cell types and diagnose disease conditions based on spatio-temporal cellular dynamics [41]. In these systems, neural networks analyze time-resolved holographic phase data to extract dynamic morphological signatures associated with specific biological states, highlighting the potential of AI-enhanced DHM as a powerful tool for label-free biomedical diagnostics.

Collectively, these studies demonstrate that deep learning can transform digital holographic microscopy from a purely imaging modality into a comprehensive platform for automated cellular analysis and disease detection. By combining quantitative phase information with data-driven pattern recognition, AI-enabled DHM systems provide a pathway toward high-throughput, label-free phenotyping of living cells.

4.6. Toward unified AI-driven quantitative phenotyping

Although the biological targets discussed above—RBCs, cancer cells, and cardiomyocytes—differ in structure and function, the underlying workflow of AI-driven DHM is remarkably unified. Each application involves three stages: hologram acquisition, where an interference pattern encodes amplitude and phase; AI-based phase reconstruction, producing clean quantitative phase maps; and AI-assisted analysis, translating phase information into morphological or functional biomarkers. In this integrated pipeline, deep and diffusion models serve as reconstruction engines as well as feature extractors, effectively bridging optical physics with biological interpretation. Recent studies further demonstrate that deep learning can operate directly on holographic measurements to perform automated detection, segmentation, and classification of cellular structures, expanding the analytical capabilities of AI-enabled DHM.

The transition from traditional optical analysis to data-centric holography has broad implications for biomedical research. AI-DHM systems now provide interpretable, quantitative outputs such as cell dry mass, contractility curves, and morphometric descriptors that can be correlated with biochemical assays or clinical outcomes. Furthermore, combining multi-cellular data from different applications enables the creation of generalizable phase-phenotype databases, facilitating cross-domain learning and anomaly detection. Such databases could, for instance, allow a network trained on RBC morphology to be fine-tuned for cancer cell classification with minimal retraining, leveraging the common physics of holographic imaging. Recent deep-learning frameworks have demonstrated automated detection and counting of phase objects directly from raw holograms, physics-guided segmentation of individual cells, discrimination of cell death pathways such as apoptosis and necroptosis, and disease diagnosis using spatio-temporal cellular dynamics. These studies illustrate how AI-driven DHM can support a unified framework for cellular phenotyping across diverse biological contexts.

Biomedical Optics EXPRESS

The convergence of optical holography, artificial intelligence, and cellular biophysics thus defines a new frontier of label-free biomedical imaging. By quantitatively linking phase to phenotype, AI-DHM transforms holographic microscopy from a qualitative visualization tool into a scalable, quantitative biophysical platform. These advances not only improve fundamental understanding of cell physiology but also open pathways for clinical translation, as explored further in Section 5 on portable and field-deployable DHM systems. A concise overview of biological targets and corresponding AI methodologies is summarized in Table 2.

Table 2. Representative biomedical applications of AI-driven DHM across blood, cancer, and cardiac cells

Cell Type	Imaging Mode	AI Model	Task	Accuracy
RBC [7,26,30]	Time-lapse off-axis	CNN / Diffusion	Storage lesion detection	> 90% accuracy
Cancer [29,45]	Off-axis	ResNet / SSL	Cell classification	95% accuracy
Cardiomyocyte [49,50]	Time-lapse off-axis	Transfer Learning	Drug toxicity screening	Precision ≈ 93%
Phase objects [61]	Lens-based DHM	CNN	Automatic detection and counting	High detection accuracy
Cell segmentation [62]	Off-axis DHM	Physics-guided DL	Quantitative cell segmentation	Improved segmentation robustness
Cell death pathway analysis [63]	Time-lapse DHM	Deep neural network	Apoptosis vs necroptosis discrimination	High classification accuracy
Disease diagnosis from cellular dynamics [41]	Compact DHM (time-series)	Deep spatio-temporal network	Cell identification and disease diagnosis	Robust diagnostic performance

5. Toward low-cost and field-deployable AI-based DHM platforms

The integration of artificial intelligence (AI) with digital holographic microscopy (DHM) has not only improved reconstruction and analysis accuracy but has also accelerated the transition of this technology from research laboratories to portable, field-deployable platforms. Unlike conventional benchtop systems that rely on bulky optical components and high-performance computers, modern DHM can now be implemented using compact illumination modules, low-cost CMOS sensors, and embedded AI processors. This convergence of optical miniaturization and computational efficiency aligns with the broader trend in biophotonics toward point-of-care diagnostics, real-time environmental monitoring, and decentralized biomedical testing.

5.1. Compact optical and hardware design

Recent innovations in DHM system architecture have significantly reduced complexity, cost, and size while maintaining quantitative accuracy. Traditional off-axis Mach-Zehnder interferometers require multiple beam splitters, mirrors, and precision alignment, making them unsuitable for field operation. To address these limitations, several groups—including Moon and Javidi's research teams—have developed common-path and single-shot configurations using inexpensive optical components and compact illumination geometries [30,31]. In particular, Park et al. [31] demonstrated a simple, single-shot DHM using a diffusion-based unsupervised phase retrieval algorithm, eliminating the need for mechanical scanning or dual-path interferometers. This simplification not only enhances robustness but also facilitates integration with portable CMOS-based imaging chips.

Biomedical Optics EXPRESS

Furthermore, optical miniaturization has been complemented by on-chip holography, where diffraction patterns are directly recorded on image sensors without relay optics. Such systems achieve submicron phase sensitivity with milliwatt-level light sources and millimeter-scale optical paths. In this context, computational reconstruction methods—including AI-based approaches—may potentially enable more robust phase retrieval under non-ideal imaging conditions. This capability could facilitate the use of reduced-coherence illumination sources, such as LEDs, in compact DHM platforms for low-cost biomedical devices and educational settings. However, the effectiveness of quantitative phase retrieval under such illumination conditions remains dependent on system design, interference visibility, and signal-to-noise characteristics. These compact designs drastically reduce power consumption and alignment sensitivity, key prerequisites for long-term field deployment. The integration of optics, embedded processing, and connectivity for portable AI-DHM platforms is outlined in Fig. 8.

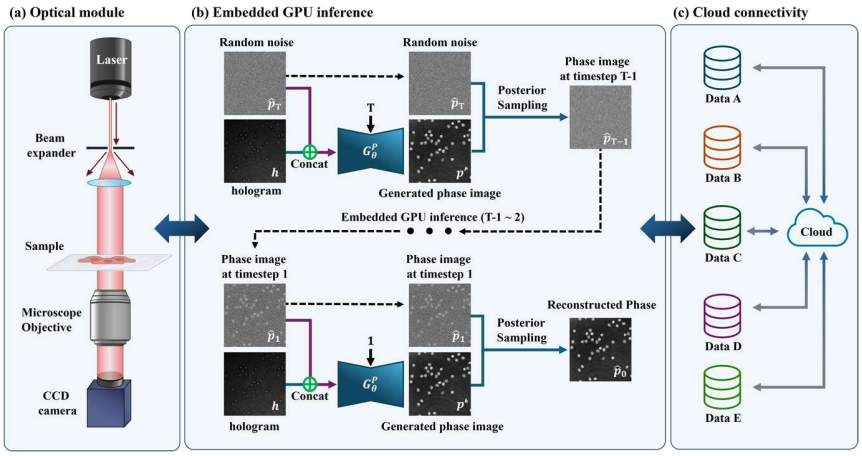

Fig. 8. Conceptual schematic of a field-deployable AI-DHM system [29–31,45]. The platform integrates (a) compact optical module, (b) embedded GPU inference, and (c) cloud connectivity for distributed data analysis.

5.2. *Edge-AI and real-time holographic analytics*

To fully realize field deployability, holographic reconstruction and analysis must occur in real time on embedded hardware [64–69]. Recent progress in edge-AI computing has made it feasible to perform deep neural network inference directly on low-power platforms such as NVIDIA Jetson, Google Coral, and ARM-based systems. Moon et al. [30] implemented a holographic sensor-integrated AI system that performs both phase reconstruction and red blood cell (RBC) quality classification on a compact embedded GPU. Their approach employed model quantization and pruning to reduce memory usage and achieve processing speeds exceeding 30 frames per second while maintaining quantitative fidelity comparable to high-end workstation implementations. The combination of optimized hardware and diffusion/self-supervised learning enables autonomous operation without cloud connectivity—a crucial requirement for remote medical diagnostics and resource-limited environments.

Edge-AI holography not only accelerates processing but also enhances data privacy and energy efficiency. Instead of transferring large holographic datasets to remote servers, the entire inference pipeline runs locally, dramatically reducing latency and network dependency. This feature is particularly valuable for applications in blood transfusion centers, rural clinics, and disaster-response settings, where internet access may be limited. In addition, the integration of compact optics with embedded computation transforms DHM from a purely imaging device into

Biomedical Optics EXPRESS

a smart biophotonic sensor, capable of on-board decision-making and adaptive measurement control.

5.3. High-throughput and flow-based implementations

Beyond static microscopy, DHM can be combined with microfluidic flow systems to achieve continuous and automated cell analysis. AI-enhanced holographic flow cytometry merges the strengths of digital holography—label-free, three-dimensional imaging—with AI-driven classification for high-throughput screening. Jaferzadeh et al. [45] and Rehman et al. [29] demonstrated the feasibility of flow-based holographic cytometry using deep and self-supervised learning for the classification of elliptical cancer cells. The AI algorithms maintained high accuracy despite motion blur and defocus inherent to flow systems, underscoring the robustness of data-driven phase interpretation. The throughput T of such systems can be expressed as:

$$T = \frac{N_{cells}}{t_{process}}, \tag{8}$$

where N_{cells} is the number of analyzed cells and $t_{process}$ refers to the total processing time, encompassing acquisition, reconstruction, and analysis. With optimized GPU inference and lightweight models, throughput values exceeding 10^4 cells per second have been reported [29,45]. These platforms hold promise for liquid biopsy, environmental biosensing, and pathogen detection, where large populations of microorganisms or cells must be characterized rapidly and without labeling.

In future implementations, flow-based AI-DHM could serve as the backbone of automated tele-biological laboratories, combining cloud data aggregation with local inference nodes. Such distributed networks would allow continuous monitoring of biological samples, similar to Internet-of-Things (IoT) architectures in industrial sensing. For example, a portable holographic cytometer equipped with edge-AI reconstruction could be deployed in mobile clinics to monitor blood quality or in water systems to detect pathogenic contamination in real time. The performance improvement achieved by AI integration in flow-based DHM is summarized in Table 3.

Table 3. Comparison of throughput and latency in conventional and AI-enhanced flow-based DHM systems

System Type	AI Integration	Throughput (cells s^{-1})	Latency (ms $frame^{-1}$)	Key Feature
Conventional DHM [7]	None	$10^2 – 10^3$	> 100	Manual analysis
AI-Enhanced Flow DHM [29]	Deep CNN	10^4	< 40	Real-time classification
Edge-AI DHM Cytometer [45]	Lightweight Diffusion	$\geq 10^4$	< 20	On-device inference

5.4. Toward global accessibility and clinical translation

The miniaturization of DHM and the integration of AI analytics directly address the global need for accessible biomedical technologies. Conventional optical instruments are often prohibitively expensive and require skilled operators, whereas portable AI-DHM platforms can deliver quantitative, automated, and reproducible analysis without extensive user training. The modular nature of these systems allows adaptation to different diagnostic contexts—ranging from hematology and oncology to cardiotoxicity and infectious disease monitoring.

However, the path toward clinical translation involves additional challenges: robust calibration across devices, standardized evaluation metrics, and compliance with medical regulations. Efforts to establish open-access holographic datasets and benchmarking frameworks will be critical to validate AI performance across institutions. Furthermore, physics-informed and explainable

Biomedical Optics EXPRESS

AI models could improve interpretability, helping clinicians understand how morphological or phase-based features correlate with disease states. Addressing these challenges will ensure that AI-driven DHM evolves into a trustworthy, clinically relevant imaging modality rather than a purely research-oriented technology.

In summary, recent developments in optical design, embedded computation, and AI model optimization have transformed DHM into a scalable and deployable platform. From single-shot, LED-based imaging [31] to edge-AI holographic sensors [30] and flow-based cytometers [29,45], these innovations collectively enable real-time, label-free, and cost-effective cellular analysis. By merging deep generative and diffusion models with compact optical hardware, AI-DHM systems promise to democratize quantitative phase imaging for applications far beyond the traditional laboratory—bridging physics, computation, and healthcare accessibility on a global scale.

6. Challenges and future perspectives

Despite remarkable progress in AI-driven digital holographic microscopy (AI-DHM), several challenges remain before the technology can be fully established as a clinical or industrial standard. Many of these limitations—as summarized in Table 4—originate from the quality and variability of holographic datasets. Deep and diffusion models require diverse and well-curated training data, yet most holographic measurements are produced in highly controlled laboratory environments with unique illumination coherence, sensor characteristics, and optical path configurations. Such variability often hinders model generalization across instruments and institutions. Establishing open-access holographic databases, standardized acquisition protocols, and unified metadata conventions will therefore be essential for improving reproducibility and enabling cross-institutional benchmarking.

Table 4. Summary of major challenges facing AI-integrated DHM and prospective research directions

Category	Current Limitation	Promising Solution
Data standardization	Limited open datasets	Shared holographic database initiatives
Domain adaptation	Setup-specific bias	Physics-informed / transfer learning
Interpretability	Black-box models	Explainable AI & PINNs
Computational cost	High power consumption	Edge-AI compression & quantization
Clinical validation	Lack of benchmarks	Multi-institutional studies

Another important obstacle is the domain-specific nature of AI-DHM models. Networks trained under a single wavelength, magnification, or sample type often degrade when applied to different imaging conditions. Approaches such as domain adaptation, transfer learning, and physics-guided regularization can help mitigate this limitation by embedding optical constraints directly into the learning process. Self-supervised learning and diffusion priors [29,31] are particularly promising because they reduce reliance on labeled datasets and adapt more flexibly to unseen holographic domains.

Interpretability presents an additional challenge. Although deep learning models have achieved impressive performance in phase reconstruction and cellular phenotyping, their internal decision processes remain difficult to understand. In biomedical applications—where reconstruction accuracy may influence diagnostic outcomes—transparent AI is essential. Emerging explainable AI (XAI) approaches, including saliency mapping and attention-based visualization, can help illuminate how networks extract structural or biophysical information from holograms. Physics-informed neural networks (PINNs) that incorporate Fresnel propagation or transport-of-intensity constraints [8,38] further enhance interpretability by linking data-driven inference with known optical physics.

Biomedical Optics EXPRESS

Scalability is another consideration, especially for high-throughput or flow-based holographic platforms. Although compact embedded systems have demonstrated real-time inference [30], processing large datasets from multi-sensor arrays or microfluidic cytometers requires more efficient neural architectures. Model compression, quantization, and neural architecture search (NAS) will play increasingly important roles in balancing computational cost and accuracy in portable or continuously operating systems.

Finally, transitioning AI-DHM to clinical practice requires rigorous validation and regulatory compliance. Quantitative performance metrics—phase accuracy, signal-to-noise ratio, and classification reproducibility—must be standardized and evaluated under realistic clinical conditions. Close collaboration between engineers, clinicians, and regulatory agencies will be essential to advance AI-DHM from laboratory demonstrations to certified diagnostic platforms.

Looking ahead, the convergence of AI, compact optics, and cloud-connected infrastructures will enable distributed holographic networks capable of continuous monitoring and remote analysis. Future systems may incorporate multimodal sensing—combining DHM with fluorescence, Raman, or hyperspectral imaging—to deliver richer structural and molecular insight. Ultimately, advances in physics-informed learning, edge computing, and standardized data ecosystems will shape the next generation of robust, interpretable, and globally accessible holographic microscopy technologies.

7. Conclusion

Artificial intelligence has fundamentally reshaped computational workflows in digital holographic microscopy, transforming it from a computationally intensive laboratory technique into an intelligent and increasingly automated imaging framework capable of both efficient phase reconstruction and quantitative cellular analysis. Through the integration of supervised, hybrid physics-guided, generative, and diffusion-based learning models, AI-DHM achieves rapid, artifact-suppressed phase reconstruction and accurate phenotypic analysis across diverse cell types, including red blood cells, cancer cells, and cardiomyocytes. Coupled with advances in compact optical design and real-time edge-AI-enabled holographic sensing platforms, these systems now enable fast, label-free quantitative imaging suitable for portable diagnostic environments. Future progress will depend on building standardized datasets, developing interpretable and physics-consistent learning frameworks, and validating performance through multi-institutional collaboration. As these challenges are met, AI-driven DHM is poised to become a cornerstone of next-generation biomedical imaging—bridging the precision of quantitative optics with the intelligence of machine learning to deliver accessible and trustworthy diagnostics worldwide.

Funding. National Research Foundation of Korea (RS-2023-00253613).

Disclosures. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

References

1. C. Boudreau, T.-L. Wee, Y.-R. Duh, *et al.*, "Excitation light dose engineering to reduce photo-bleaching and photo-toxicity," Sci. Rep. **6**(1), 30892 (2016).
2. J. Icha, M. Weber, J. C. Waters, *et al.*, "Phototoxicity in live fluorescence microscopy, and how to avoid it," BioEssays **39**(8), 1700003 (2017).
3. S. M. Hickey, B. Ung, C. Bader, *et al.*, "Fluorescence microscopy—an outline of hardware, biological handling, and fluorophore considerations," Cells **11**(1), 35 (2021).
4. J. Kwon, M. S. Elgawish, S. Shim, *et al.*, "Bleaching-resistant super-resolution fluorescence microscopy," Adv. Sci. **9**(9), 2101817 (2022).
5. W. Xu, M. H. Jericho, I. A. Meinertzhagen, *et al.*, "Digital in-line holography for biological applications," Proc. Natl. Acad. Sci. **98**(20), 11301–11305 (2001).

Biomedical Optics EXPRESS

6. I. Moon and B. Javidi, "3-D Visualization and Identification of Biological Microorganisms Using Partially Temporal Incoherent Light In-Line Computational Holographic Imaging," IEEE Trans. Med. Imaging **27**(12), 1782–1790 (2008).
7. K. Jaferzadeh, I. Moon, M. Bardyn, *et al.*, "Quantification of stored red blood cell fluctuations by time-lapse holographic cell imaging," Biomed. Opt. Express **9**(10), 4714–4729 (2018).
8. I. Moon, K. Jaferzadeh, Y. Kim, *et al.*, "Noise-free quantitative phase imaging in Gabor holography with conditional generative adversarial network," Opt. Express **28**(18), 26284–26301 (2020).
9. E. Cuche, P. Marquet, C. Depeursinge, *et al.*, "Simultaneous amplitude-contrast and quantitative phase-contrast microscopy by numerical reconstruction of Fresnel off-axis holograms," Appl. Opt. **38**(34), 6994–7001 (1999).
10. B. Rappaz, P. Marquet, E. Cuche, *et al.*, "Measurement of the integral refractive index and dynamic cell morphometry of living cells with digital holographic microscopy," Opt. Express **13**(23), 9361–9373 (2005).
11. B. Kemper and G. V. Bally, "Digital holographic microscopy for live cell applications and technical inspection," Appl. Opt. **47**(4), A52–A61 (2008).
12. D. Roitshtain, L. Wolbromsky, E. Bal, *et al.*, "Quantitative phase microscopy spatial signatures of cancer cells," Cytometry Pt A **91**(5), 482–493 (2017).
13. B. Javidi, A. Carnicer, A. Anand, *et al.*, "Roadmap on digital holography," Opt. Express **29**(22), 35078–35118 (2021).
14. A. Anand, I. Moon, and B. Javidi, "Automated Disease Identification With 3-D Optical Imaging: A Medical Diagnostic Tool," Proc. IEEE **105**(5), 924–946 (2017).
15. B. Rappaz, A. Barbul, Y. Emery, *et al.*, "Comparative study of human erythrocytes by digital holographic microscopy, confocal microscopy, and impedance volume analyzer," Cytometry Pt A **73A**(10), 895–903 (2008).
16. F. Yi, I. Moon, Y. H. Lee, *et al.*, "Three-dimensional counting of morphologically normal human red blood cells via digital holographic microscopy," J. Biomed. Opt. **20**(1), 016005 (2015).
17. K. Jaferzadeh and I. Moon, "Human red blood cell recognition enhancement with three-dimensional morphological features obtained by digital holographic imaging," J. Biomed. Opt. **21**(12), 126015 (2016).
18. M. Bardyn, B. Rappaz, K. Jaferzadeh, *et al.*, "Red blood cells ageing markers: a multi-parametric analysis," Blood Transfusion **15**(3), 239–248 (2017).
19. G. D. Bhabhor, C. Patel, N. Chhillar, *et al.*, "Geometrical characterization of healthy red blood cells using digital holographic microscopy and parametric shape models for biophysical studies and diagnostic applications," J. Phys. D: Appl. Phys. **57**(35), 355401 (2024).
20. K. Jeong, J. J. Turek, D. D. Nolte, *et al.*, "Fourier-domain digital holographic optical coherence imaging of living tissue," Appl. Opt. **46**(22), 4999–5008 (2007).
21. L. Guo, X. Wang, P. Han, *et al.*, "Observation of dehydration dynamics in biological tissues with terahertz digital holography," Appl. Opt. **56**(13), F173–F178 (2017).
22. N. T. Shaked, L. L. Satterwhite, N. Bursac, *et al.*, "Whole-cell-analysis of live cardiomyocytes using wide-field interferometric phase microscopy," Biomed. Opt. Express **1**(2), 706–719 (2010).
23. E. Ahamadzadeh, K. Jaferzadeh, S. Park, *et al.*, "Automated analysis of human cardiomyocytes dynamics with holographic image-based tracking for cardiotoxicity screening," Biosens. Bioelectron. **195**, 113570 (2022).
24. K. Jaferzadeh, S.-H. Hwang, I. Moon, *et al.*, "No-search focus prediction at the single cell level in digital holographic imaging with deep convolutional neural network," Biomed. Opt. Express **10**(8), 4276–4289 (2019).
25. S. Park, Y. Kim, I. Moon, *et al.*, "Automated phase unwrapping in digital holography with deep learning," Biomed. Opt. Express **12**(11), 7064–7081 (2021).
26. E. Kim, S. Park, S. Hwang, *et al.*, "Deep learning-based phenotypic assessment of red cell storage lesions for safe transfusions," IEEE J. Biomed. Health Inform. **26**(3), 1318–1328 (2022).
27. S. Park, Y. Kim, and I. Moon, "Fast automated quantitative phase reconstruction in digital holography with unsupervised deep learning," Opt. Laser. Eng. **167**, 107624 (2023).
28. S. Park, Y. Kim, and I. Moon, "Automated phase reconstruction and super-resolution with deep learning in digital holography," Opt. Laser Technol. **176**, 111030 (2024).
29. A. Rehman, H. An, S. Park, *et al.*, "Automated classification of elliptical cancer cells with stain-free holographic imaging and self-supervised learning," Opt. Laser Technol. **174**, 110646 (2024).
30. S. Park, H. An, A. Rehman, *et al.*, "A Holographic Sensor-Integrated Deep Learning Framework for Noninvasive Assessment of Stored Red Blood Cell Quality," Advanced Sensor Research **4**(12), e00073 (2025).
31. S. Park, J. Park, Y. Kim, *et al.*, "Simple and practical single-shot digital holography based on unsupervised diffusion model," Engineering Applications of Artificial Intelligence **163**, 112970 (2026).
32. G. Barbastathis, A. Ozcan, G. Situ, *et al.*, "On the use of deep learning for computational imaging," Optica **6**(8), 921–943 (2019).
33. Z. Li, F. Liu, W. Yang, *et al.*, "A survey of convolutional neural networks: analysis, applications, and prospects," IEEE Trans. Neural Netw. Learning Syst. **33**(12), 6999–7019 (2022).
34. I. Goodfellow, J. Pouget-Abadie, M. Mirza, *et al.*, "Generative adversarial networks," Commun ACM **63**(11), 139–144 (2020).
35. J. Ho, A. Jain, and P. Abbeel, "Denoising diffusion probabilistic models," Advances in Neural Information Processing Systems **33**, 6840–6851 (2020).
36. Z. Xiao, K. Kreis, and A. Vahdat, "Tackling the generative learning trilemma with denoising diffusion GANs," arXiv (2021).

Biomedical Optics EXPRESS

37. A. Jaiswal, A. R. Babu, M. Z. Zadeh, *et al.*, "A survey on contrastive self-supervised learning," Technologies **9**(1), 2 (2020).
38. Y. Rivenson, Y. Zhang, H. Günaydın, *et al.*, "Phase recovery and holographic image reconstruction using deep learning in neural networks," Light: Sci. Appl. **7**(2), 17141 (2017).
39. Y. Wu, Y. Rivenson, Y. Zhang, *et al.*, "Extended depth-of-field in holographic imaging using deep-learning-based autofocusing and phase recovery," Optica **5**(6), 704–710 (2018).
40. Z. Ren, Z. Xu, and E. Y. Lam, "End-to-end deep learning framework for digital holographic reconstruction," Adv. Photonics **1**(01), 1 (2019).
41. T. O'Connor, A. Anand, B. Andemariam, *et al.*, "Deep learning-based cell identification and disease diagnosis using spatio-temporal cellular dynamics in compact digital holographic microscopy," Biomed. Opt. Express **11**(8), 4491–4508 (2020).
42. P. M. Douglass, T. O'Connor, and B. Javidi, "Automated sickle cell disease identification in human red blood cells using a lensless single random phase encoding biosensor and convolutional neural networks," Opt. Express **30**(20), 35965–35977 (2022).
43. K. Wang, L. Song, C. Wang, *et al.*, "On the use of deep learning for phase recovery," Light: Sci. Appl. **13**(1), 4 (2024).
44. F. Yi, I. Moon, B. Javidi, *et al.*, "Cell morphology-based classification of red blood cells using holographic imaging informatics," Biomed. Opt. Express **7**(6), 2385–2399 (2016).
45. K. Jaferzadeh, S. Son, A. Rehman, *et al.*, "Automated stain-free holographic image-based phenotypic classification of elliptical cancer cells," Advanced Photonics Research **4**(1), 2200043 (2023).
46. T. Chen, S. Kornblith, M. Norouzi, *et al.*, "A simple framework for contrastive learning of visual representations," in *International conference on machine learning*, (PmLR, 2020), 1597–1607.
47. J. Zbontar, L. Jing, I. Misra, *et al.*, "Barlow twins: Self-supervised learning via redundancy reduction," in *International conference on machine learning*, (PMLR, 2021), 12310–12320.
48. M. Caron, I. Misra, and J. Mairal, "Unsupervised learning of visual features by contrasting cluster assignments," Advances in Neural Information Processing Systems **33**, 9912–9924 (2020).
49. I. Moon, K. Jaferzadeh, E. Ahmadzadeh, *et al.*, "Automated quantitative analysis of multiple cardiomyocytes at the single-cell level with three-dimensional holographic imaging informatics," J. Biophotonics **11**(12), e201800116 (2018).
50. E. Ahmadzadeh, S. Park, Y. Kim, *et al.*, "Automated phenotypic analysis and classification of drug-treated cardiomyocytes via synergized time-lapse holographic imaging and deep learning," Computer Methods and Programs in Biomedicine **269**, 108890 (2025).
51. J. Quiroga and E. Bernabeu, "Phase-unwrapping algorithm for noisy phase-map processing," Appl. Opt. **33**(29), 6725–6731 (1994).
52. M. Pritt and J. Shipman, "Least-squares two-dimensional phase unwrapping using FFT's," IEEE Trans. Geosci. Remote Sensing **32**(3), 706–708 (1994).
53. M. A. Herráez, D. R. Burton, M. J. Lalor, *et al.*, "Fast two-dimensional phase-unwrapping algorithm based on sorting by reliability following a noncontinuous path," Appl. Opt. **41**(35), 7437–7444 (2002).
54. T. Colomb, E. Cuche, F. Charrière, *et al.*, "Automatic procedure for aberration compensation in digital holographic microscopy and applications to specimen shape compensation," Appl. Opt. **45**(5), 851–863 (2006).
55. D. Gabor, "A new microscopic principle," Nature **161**(4098), 777–778 (1948).
56. G. Koren, F. Polack, and D. Joyeux, "Iterative algorithms for twin-image elimination in in-line holography using finite-support constraints," J. Opt. Soc. Am. A **10**(3), 423–433 (1993).
57. T. Nakamura, K. Nitta, and O. Matoba, "Iterative algorithm of phase determination in digital holography for real-time recording of real objects," Appl. Opt. **46**(28), 6849–6853 (2007).
58. J. Di, J. Wu, K. Wang, *et al.*, "Quantitative phase imaging using deep learning-based holographic microscope," Front. Phys. **9**, 651313 (2021).
59. Z. Li, J. Sun, Y. Fan, *et al.*, "Deep learning assisted variational Hilbert quantitative phase imaging," Opto-Electronic Sci. **2**(4), 220023 (2023).
60. J. Zhao, L. Liu, T. Wang, *et al.*, "Quantitative phase imaging of living red blood cells combining digital holographic microscopy and deep learning," J. Biophotonics **16**(10), e202300090 (2023).
61. C. Trujillo and J. Garcia-Sucerquia, "Automatic detection and counting of phase objects in raw holograms of digital holographic microscopy via deep learning," Opt. Lasers Eng. **120**, 13–20 (2019).
62. Z. Li, H. Gu, L. Lu, *et al.*, "DL-CSPF: deep-learning-based cell segmentation with a physical framework for digital holographic microscopy," Appl. Opt. **64**(7), B20–B30 (2025).
63. J. Verduijn, L. Van der Meeren, D. V. Krysko, *et al.*, "Deep learning with digital holographic microscopy discriminates apoptosis and necroptosis," Cell Death Discovery **7**(1), 229 (2021).
64. A. C. Sobieranski, F. Inci, H. C. Tekin, *et al.*, "Portable lensless wide-field microscopy imaging platform based on digital inline holography and multi-frame pixel super-resolution," Light: Sci. Appl. **4**(10), e346 (2015).
65. L. Shi, B. Li, C. Kim, *et al.*, "Towards real-time photorealistic 3D holography with deep neural networks," Nature **591**(7849), 234–239 (2021).
66. Z.-S. Li, C. Liu, X.-W. Li, *et al.*, "Real-time holographic camera for obtaining real 3D scene hologram," Light: Sci. Appl. **14**(1), 74 (2025).

Biomedical Optics EXPRESS

67. S. Rawat, S. Komatsu, A. Markman, *et al.*, "Compact and field-portable 3D printed shearing digital holographic microscope for automated cell identification," Appl. Opt. **56**(9), D127–D133 (2017).
68. T. O'Connor, J.-B. Shen, B. T. Liang, *et al.*, "Digital holographic deep learning of red blood cells for field-portable, rapid COVID-19 screening," Opt. Lett. **46**(10), 2344–2347 (2021).
69. T. O'Connor, A. Anand, and B. Javidi, "Field-portable microsphere-assisted high resolution digital holographic microscopy in compact and 3D-printed Mach-Zehnder Interferometer," OSA Continuum **3**(4), 1013–1020 (2020).