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AI-Assisted Microscopy for Infection Biology: Advances in High-Content Imaging of Host-Pathogen Interactions

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Advances in high-content microscopy and artificial intelligence (AI) are transforming the quantitative study of infection biology. Automated imaging platforms now enable rapid, large-scale acquisition of host-pathogen interactions across thousands of cells and multiple experimental conditions. When combined with AI-based segmentation, these workflows extract infection-relevant features such as pathogen load, intracellular localization, and host response markers at single-cell resolution. Deep-learning models have proven especially powerful, outperforming classical threshold-based methods under different imaging conditions, reducing reliance on manual annotation, and detecting rare infection outcomes. Beyond robust image analysis, these approaches generate scalable and reproducible datasets that can be integrated with computational modelling and systems biology, providing predictive insight into infection dynamics. This review highlights recent progress in AI-assisted microscopy for bacterial infection and outlines future directions toward multimodal integration, clinical translation, and open-source tool development.

Keywords: artificial intelligence, machine learning, deep learning, host-pathogen interactions, single-cell biology, cell-to-cell variability, cellular heterogeneity, infection biology.

1. Introduction

The study of host-pathogen interactions has been revolutionized by microscopy. From early fluorescence imaging of infected cells to the development

of automated high-content systems, infection biology has increasingly relied on quantitative visualization [12, 23, 40, 43]. Yet, traditional manual annotation and threshold-based image analysis [33] remain limiting. They are labour-intensive, prone to operator bias, and can be poorly scalable when dealing with tens of thousands of cells across multiple infection conditions [8, 29].

Artificial intelligence (AI), particularly deep learning (DL), a class of algorithms based on multi-layered neural networks capable of automatically extracting complex patterns from large datasets, now provides transformative solutions [27, 31, 44]. By combining automated high-content imaging with advanced image analysis workflows, researchers can extract infection-relevant features, including pathogen load, subcellular localization, and host cell responses at single-cell resolution and across entire experiments [19, 28]. Importantly, AI-based models outperform classical approaches under diverse imaging conditions, reduce the need for manual annotation, and enable detection of rare infection outcomes [14, 20, 39]. This review highlights recent advances in AI-assisted microscopy for bacterial infection biology, building on pioneering work in host–pathogen imaging and linking these efforts to broader computational sciences (Fig. 1 and Table 1).

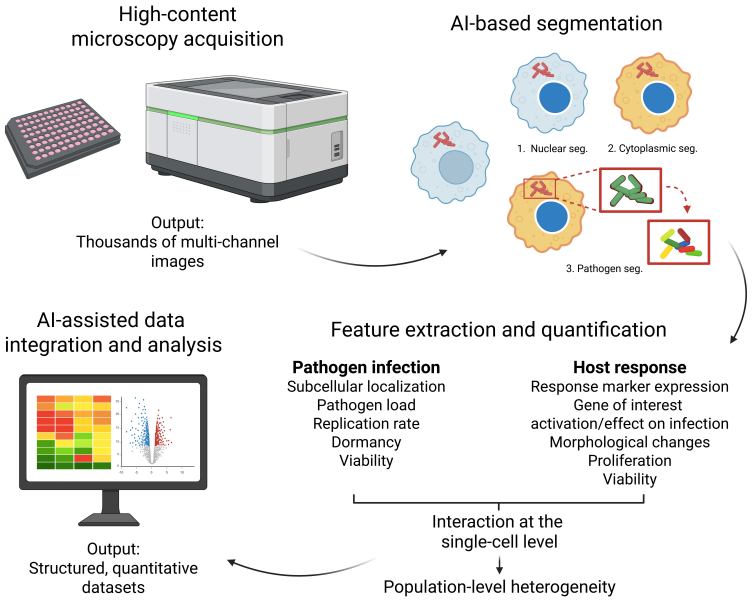

FIG. 1. Workflow of AI-assisted microscopy for infection biology. High-content microscopy generates thousands of multi-channel images from infected cells. AI-based segmentation (e.g., CNNs, Omnipose, DeepBacs) identifies host nuclei, cytoplasm, and intracellular bacteria. Single-cell feature extraction quantifies pathogen load, localization, and host responses. Integrated features reveal infection heterogeneity and can inform computational models of infection dynamics.

TABLE 1. Summary of discussed technologies and applications.

Category	Representative tools	Purpose	Key advantage	References
Methods and technologies				
Classical image analysis	ImageJ, CellProfiler	Rule-based segmentation and quantification	Simple, widely used, reproducible	[12, 29, 33, 37]
High-content microscopy	Automated HCS, live-cell imaging	Large-scale acquisition of infection datasets	High throughput; supports time-lapse assays	[4, 24, 30]
Deep learning segmentation	HRMAn, DeepBacs, Omnipose	AI-based detection of host cells and bacteria	Robust to morphology; high accuracy	[14, 19, 20, 31, 39]
AI-enhanced microscopes	AI autofocus, denoising, PhaseStain, BlurryScope, pySTED	Real-time correction and enhancement, super-resolution	Improves quality from low-cost or noisy images	[5, 10, 17, 36]
Applications				
Single-cell infection heterogeneity	DL phenotype mapping	Quantify infection outcomes per cell	Detects rare events; resolves heterogeneity	[28, 45]
Tracking & feature extraction	da_Tracker, RABiTPy	Tracking of bacteria, phagosomes, cell states	High-throughput, automated quantification	[2, 38]
Systems biology integration	Imaging + scRNA-seq, spatial omics	Link phenotypes to gene regulatory programs	Multimodal mechanistic insight	[6, 13, 16, 46]
Clinical AI imaging	DL for TB CT; imaging mass cytometry	Diagnosis and clinical outcome prediction	Non-invasive, scalable	[47, 48]

2. High-content microscopy in infection research

Automated microscopy platforms enable systematic acquisition of hundreds of fields or entire multi-well plates in minutes, capturing tens of thousands of host and pathogen cells. Importantly, these systems also support longitudinal live-cell assays, allowing researchers to track infection trajectories and cell fate decisions in real time. Such time-resolved imaging is particularly powerful for studying dynamic host responses, pathogen replication, and the heterogeneity of host-pathogen interactions across individual cells [7]. Illustrating these applications, Batani *et al.* [4] developed a high-content adhesion/invasion inhibition assay to assess the functionality of *Shigella*-specific antibodies, showing how automated imaging can quantify pathogen adhesion and host cell entry in a reproducible and scalable fashion [4]. Similarly, high-throughput screens have been applied to bacterial and viral pathogens to capture phenotypic diversity and responses to treatment, in a more physiological context [24, 30, 45]. Beyond infection biology, such platforms have also been central to cell profiling and drug discovery, underscoring their versatility and robustness [8].

Raw image acquisition is only the first step of the analysis pipeline. Infection biology presents unique computational challenges: bacterial morphologies vary widely, host cells exhibit diverse responses, and infections are heterogeneous both across and within populations [7]. Classical image analysis approaches, often based on local and global intensity thresholding and handcrafted morphological features can struggle with such complexity [33, 37]. These limitations call for robust, generalizable image analysis strategies, precisely the areas where AI-based methods excel [44].

3. AI-driven image segmentation and feature extraction

Classical image analysis pipelines long served as the workhorse of infection microscopy, relying on combinations of intensity thresholds, handcrafted filters, and morphological operations to segment host cells and detect intracellular pathogens [12, 23, 29, 33, 37]. While these approaches proved effective in controlled imaging conditions and remain widely used in platforms such as ImageJ and CellProfiler, their performance can suffer in the face of biological heterogeneity. Individual bacteria may be small, dim, or clustered near host structures or inside different host cell compartments, while host cells themselves vary greatly in size, shape, and fluorescence signal. Under such conditions, rule-based methods tend to misclassify pixels, merge or split objects incorrectly, requiring extensive manual parameter tuning.

DL models have demonstrated substantial improvements in robustness and accuracy by overcoming these constraints. Instead of relying on fixed rules,

DL architectures such as convolutional neural networks (CNNs) learn hierarchical features directly from training data, making them resilient to imaging variability. Early proof-of-concept work by Fisch *et al.* [19] showed how CNNs-enabled HRMAn (Host Response to Microbe Analysis) could simultaneously detect bacteria and host compartments in complex infection images as well as recognize, classify and quantify pathogen killing, replication and cellular defence responses, without relying on researcher-based assumptions, paving the way for more generalizable approaches. Building on this foundation, Spahn *et al.* [39] introduced DeepBacs, an open-source multi-task DL pipeline capable of bacterial segmentation and phenotyping across diverse datasets, while Cutler *et al.* [14] developed Omnipose, a morphology-independent segmentation tool that excels in recognizing bacteria of varying shapes, including filamentous or irregular forms where traditional algorithms often fail. Complementing these tools, Fisch *et al.* [20] released HRMAn 2.0, a generalizable AI-driven analysis platform for broad host-pathogen interactions, supporting multiple host cell types and pathogens like *Toxoplasma gondii*, *Chlamydia trachomatis* and the fungal *Cryptococcus neoformans*. More recently, López-Jiménez *et al.* [28] combined high-resolution microscopy with DL to reveal heterogeneity during *Shigella* infection, identifying subpopulations of both bacteria and host cells that would be undetectable by manual annotation.

AI algorithms and pipelines continue to be refined, including methods that enhance image resolution through post-processing, making it possible to extract quantitative data even from lower-cost microscopes [1, 34, 35, 42]. In recent years, several open-source Python packages have also emerged to simplify bacterial segmentation, tracking, analysis, and visualization within unified, scriptable interfaces. Examples include da_Tracker [2], a near-automated workflow for high-throughput cell and phagosome tracking, and RABiTPy [38], which offers a fully integrated pipeline for bacterial tracking and analysis. A notable development is the integration of AI directly into modern imaging platforms. Confocal and high-content systems now embed modules for autofocus, denoising, deblurring, and even real-time segmentation [10, 17, 36]. These "smart microscopes" open the door to adaptive acquisition, where instruments dynamically adjust scanning to capture rare infection events or resolve subtle intracellular features.

Together, these advances demonstrate how AI pipelines are evolving from task-specific solutions toward flexible, general-purpose frameworks and "smart microscopes" for host-pathogen imaging.

4. Quantifying infection heterogeneity at single-cell resolution

While high-content microscopy and AI pipelines enable large-scale and accurate segmentation, the real power of these approaches lies in their ability to

resolve infection heterogeneity at the single-cell level. Infection outcomes are not uniform: within the same population of genetically-identical cells, some may resist invasion, others may clear intracellular bacteria, while another fraction supports extensive pathogen replication leading to cell death or, in some cases, persistence [3, 11, 15, 18, 22, 26, 32, 41]. Capturing this spectrum of outcomes is crucial for understanding infection dynamics and host susceptibility [6].

Quantitative image analysis has already demonstrated its value in this context. For example, Voznica *et al.* [45] used image analysis and modelling to investigate host epithelium susceptibility to *Salmonella* infection. Their work demonstrated that single-cell phenotypic features of host cells, namely morphology, local crowding and cholesterol signalling may predict infection probability. Similarly, López-Jiménez *et al.* [28] highlighted how DL can capture both bacterial and host heterogeneity during *Shigella* infection, linking divergent outcomes to DNA and protein synthesis, host morphological changes, and type III secretion system activity in bacteria, thereby resolving infection trajectories at an unprecedented scale. More broadly, AI approaches have enabled the identification of rare events such as failed invasion attempts or rapid bacterial clearance, events often overlooked in bulk analyses but critical for understanding protective immunity [7]. By moving from bulk averages to single-cell resolution, AI-driven microscopy turns heterogeneity into a quantifiable feature, bridging infection biology with predictive modelling and systems-level understanding of host–pathogen dynamics.

5. Integration with computational modelling and systems biology

AI-assisted image analysis generates rich, quantitative datasets describing pathogen load, spatial localization, host cell state, and infection trajectories. The next frontier is to integrate these phenotypic measurements with mathematical and computational models. Such integration enables the formulation of predictive frameworks that go beyond descriptive imaging, providing mechanistic insight into infection dynamics.

Single-cell features derived from imaging can inform agent-based or statistical models of infection spread, allowing predictions of how outcomes shift under different host or bacterial genotypes. Similarly, imaging-derived parameters such as intracellular bacterial growth rates or clearance probabilities can be incorporated into dynamical systems models to test hypotheses about immune control or pathogen evasion strategies [21]. Caicedo *et al.* [8] outlined general strategies for image-based cell profiling, many of which can be directly adapted to the infection context.

Recent advances in multimodal single-cell analysis also provide opportunities for integration. AI-driven microscopy could be combined with transcriptomics

or proteomics [44], bridging phenotypic imaging with molecular profiling and linking cell states to underlying gene regulatory or metabolic networks. For instance, imaging mass cytometry has been used to integrate spatial proteomics with clinical phenotypes at single-cell resolution [25]. Spatial transcriptomics approaches, such as seqFISH and MERFISH, provide complementary maps of gene expression that can be correlated with infection phenotypes observed by microscopy [16, 46]. In addition, AI-assisted pipelines have begun linking live-cell imaging with single-cell RNA-seq, enabling direct connections between observed cellular behaviours and underlying transcriptional programs [9]. Together, these integrative strategies highlight how microscopy-derived phenotypes can be contextualized within broader molecular landscapes, advancing systems-level understanding of host–pathogen interactions.

Applications in infectious disease research illustrate the potential of this approach. In tuberculosis, deep learning models have been developed for automated CT analysis, predicting lesion dynamics and treatment outcomes, while highlighting challenges of generalization, interpretability, and clinical validation [47]. Another study showed how the maximum cross-sectional area of lesions on CT could predict early therapeutic response in multidrug-resistant tuberculosis (MDR-TB), demonstrating how imaging-derived metrics can guide clinical decisions [48]. Integrative efforts are also expanding in microscopy methodology itself. Bilodeau *et al.* [5] presented pySTED, a simulation platform for super-resolution microscopy that incorporates validated models for photobleaching, point spread function, scanning dynamics, and structural realism. By generating realistic synthetic data, pySTED facilitates data augmentation, benchmarking, and reinforcement learning approaches.

Together, these cross-cutting efforts highlight how linking AI-based microscopy with systems-level models can transform infection biology, enabling predictive frameworks where cell-level measurements inform population-scale and clinical outcomes.

6. Outlook and conclusions

AI-assisted microscopy has rapidly advanced the field of infection biology, but several challenges remain. Training deep learning models still requires large, annotated datasets, which are often lacking for rare pathogens or specialized assays. Strategies such as transfer learning and synthetic data generation may help overcome this barrier [31]. Interpretability is another frontier: while CNNs excel at segmentation and feature extraction, linking features to mechanistic biological insight remains difficult. Hybrid approaches that couple AI-driven image analysis with mathematical infection models may provide a powerful route to prediction and explanation [45].

Looking ahead, future progress will depend on making AI pipelines more adaptable across pathogens, cell types, and imaging conditions, while reducing the need for retraining. Integration with multimodal single-cell omics offers the chance to link infection phenotypes with transcriptional, proteomic, and epigenetic states, providing richer insight into host–pathogen interactions. Clinical applications are another frontier, with clear potential in diagnostics, drug screening, and vaccine testing. At the same time, the expansion of open-source toolkits like DeepBacs and Omnipose, coupled with curated infection imaging datasets, will be critical for accelerating adoption and ensuring reproducibility.

By bridging microscopy with computational intelligence, infection biology is entering a new era of scalability, reproducibility, and predictive power. AI-driven approaches not only resolve the heterogeneity of infection outcomes at single-cell resolution but also generate quantitative datasets ideally suited for systems-level modelling. In this way, infection biology finds a place within the broader landscape of computational sciences, offering both a demanding test case and a fertile application domain. The convergence of AI, high-content microscopy, and systems biology holds strong promise for transformative discoveries in infection research and beyond, particularly as emerging "smart microscopes" begin to integrate AI modules directly into acquisition workflows. Extending these capabilities from general imaging tasks to pathogen-specific contexts could further accelerate discovery.

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