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INVITED REVIEW

# Artificial intelligence-powered microscopy: Transforming the landscape of parasitology

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**Abstract**

Microscopy and image analysis play a vital role in parasitology research; they are critical for identifying parasitic organisms and elucidating their complex life cycles. Despite major advancements in imaging and analysis, several challenges remain. These include the integration of interdisciplinary data; information derived from various model organisms; and data acquired from clinical research. In our view, artificial intelligence—with the latest advances in machine and deep learning—holds enormous potential to address many of these challenges. This review addresses how artificial intelligence, machine learning and deep learning have been used in the field of parasitology—mainly focused on Apicomplexan, Diplomonad, and Kinetoplastid groups. We explore how gaps in our understanding could be filled by AI in future parasitology research and diagnosis in the field. Moreover, it addresses challenges and limitations currently faced in implementing and expanding the use of artificial intelligence across biomedical fields. The necessary increased collaboration between biologists and computational scientists will facilitate understanding, development, and implementation of the latest advances for both scientific discovery and clinical impact. Current and future AI tools hold the potential to revolutionise parasitology and expand One Health principles.

KEYWORDS

AI-based diagnosis, Deep learning, Host-pathogen interactions, Image analysis, Microscopy, Parasitology

## 1 | INTRODUCTION

Microscopy is vital to parasitology, critical for understanding basic parasite biology, host–parasite interactions, pathology, antiparasitic drug resistance, and clinical diagnosis. Historically, microscopy was fundamental for the initial identification of most parasites, and revealed key steps of their complex, multihost life cycles. Parasitology is an extensive field which includes endo- and ectoparasites with the ability to infect animal, plant and fungal groups. However, for the purpose of this review, we will focus on endoparasites of clinical relevance of the Apicomplexan,

Diplomonad and Kinetoplastid groups. This includes those that are transmitted by the fecal-oral route (such as *Toxoplasma gondii*, *Giardia* spp., and *Cryptosporidium* spp.), sexually transmitted (such as *Cyclospora cayetanensis*), and vector-borne (such as *Plasmodium* spp., *Babesia* spp., *Theileria* spp., *Trypanosoma* spp., and *Leishmania* spp.). Although extensive work has been done relative to helminths, this is not the expertise of any of the authors of this work, and it is beyond the scope of this review.

Light microscopy has been the gold standard for diagnosis in endemic settings for decades. Other types of microscopy have allowed researchers to glean a deeper understanding of parasites outside the clinic. The advances in the fields of light,[1–4] electron,[5–7] scanning probe[8–11] and cryo-STEM and soft X ray tomography,[12–17] as well as clinical and in vivo imaging[18,19] have greatly increased our understanding of parasites and their hosts.

In contrast to the advances in microscopy, the adoption and generation of image analysis workflows has significantly lagged, largely due to three key factors. These are as follows: reduced funding to this specific field; lack of accessible image databases that facilitate the generation of relevant workflows (particularly, training deep learning models); and a relative lack of interdisciplinary communication and cross-training between biology and computational science experts. Reducing the gap between these two fields of expertise is paramount for parasitology for two key reasons. First, current imaging technologies produce vast amounts of data that, without the computational expertise and capacity for analysis, cannot be used to the highest potential. Second, the exponential growth of artificial intelligence (AI), including its incorporation to microscopy and image analysis, will bring about unprecedented opportunities for discovery. In this context, it is important that the scientific community is ready to exploit the potential that AI represents for research. Equally importantly for this field is that AI has the potential to streamline diagnosis and treatment in a manner that will increase accuracy and efficacy while reducing the diagnostic burden on clinicians. This will re-direct dedicated experts to other pressing tasks for disease control, such as technology development, clinical assistance, drug development, or monitoring and surveillance.

In this review, aimed at parasitologists interested in expanding their knowledge on AI or image analysis, we begin by providing an overview of what is the difference between AI, machine learning (ML) and deep learning (DL), and we give an overview of their uses and applications in imaging and image analysis. We continue by discussing the current uses of AI in parasitology following a systematic review of the existing literature. Then we explore how existing AI tools in other fields can contribute to answer outstanding questions in the field of parasitology. We conclude by discussing considerations and challenges of AI implementation in microscopy across biomedical fields, including parasitology.

## 2 | MICROSCOPY IN PARASITOLOGY RESEARCH: VALUE, CHALLENGES AND PROSPECTS WITH AI

### 2.1 | Defining AI, ML and DL

Our modern concept of AI originated in the 1950s.[20] Research in neuroscience had shown that the brain was an electrical network of neurons that communicate through all-or-nothing pulses. Key figures in this field are Warren McCulloch, Walter Pitts, and Frank Rosenblatt. After the initial 'spark' of the 1950s, the following two decades saw revolutionary innovations incorporating AI: the first industrial robot,[21] the first expert system with decision-making abilities,[22] the first 'chatbot',[23] and the Stanford Cart.[24] Since the 1990s, AI has gained huge momentum across fields of science and technology. The impact of these advances is evident in the 2024 Nobel Prizes in Physics and Chemistry.[25] In Physics, the awards recognise developments that have been fundamental for artificial neural networks, and the rapid processing of information, effective learning and memory generation (i.e. the Hopfield Network[26] and the Boltzmann Machine[27]). In Chemistry, they recognise the development and refinement of computational protein design and AlphaFold2, an AI model with the potential of predicting the structures of all identified proteins.[28]

Although AI, ML, and DL are often used interchangeably, there are important differences between these concepts (Figure 1). Overall descriptions and main concepts and algorithms of each are summarised in Table 1. A glossary of all AI-related terms used in this review is included in Table 2. Main pitfalls of AI are summarised in Table 3.

- AI is a general term for a set of technologies implemented in systems, including ML and DL, that enables computers and robots to reason, learn, and solve complex problems. AI implementation in microscopy and biomedical sciences includes hardware, software, expertise and a combination of all. Examples of applications of AI in imaging are smart sample navigation at the microscope and setting adjustment for optimal image acquisition.
- ML is a subfield of AI that enables machines to autonomously learn insights, recognise patterns from data, improve from experience, and apply such learning to make decisions.[29] Examples include training an imaging system to identify a specific cell type in

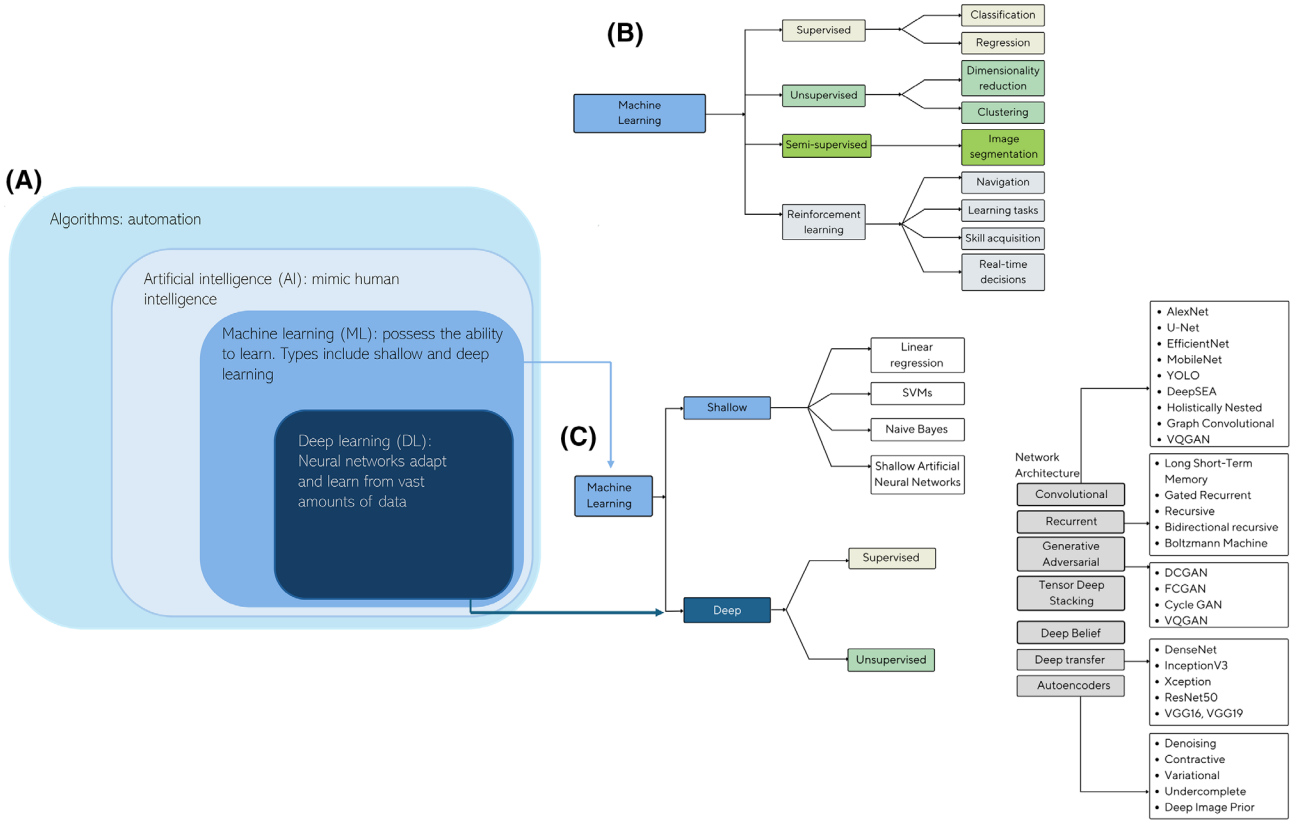


**FIGURE 1** Key subgroups in artificial intelligence. (A) Algorithms are the basis of artificial intelligence (AI) and enable automation. AI is the use of such algorithms to attempt to mimic human intelligence. Machine learning (ML) is a subgroup in AI, and deep learning (DL) is a subgroup of ML. (B) Shallow and deep ML are often categorised into 4 groups: supervised, unsupervised, semi-supervised, and reinforcement learning. Examples of applications for each are shown. (C) Common ML and DL network architectures. While more examples exist, we emphasise those mentioned in this work.

a heterogeneous sample, finding the cell, and acquiring a high-quality image of that cell. Another example of ML is training a system to segment and classify cells and structures in a way that is generalisable and reproducible.[30] Machine learning algorithms tend to be divided into four main categories based on learning methods: supervised learning, unsupervised learning, semi-supervised learning and reinforcement learning (Figure 1). Details of each are summarised in Table 1.

- DL is a type of ML that uses artificial neural networks to learn from data. Both ML and DL can be trained on labelled or unlabelled data, and both are applicable to similar tasks. However, DL often outperforms traditional ML in complex pattern recognition tasks like image classification and object detection, due to its non-linearities, and its ability to learn hierarchical representations of data. Neural networks are made up of layers of interconnected nodes, whereby specific features about the data are a collective representation learned by multiple nodes. Examples of deep learning models are convolutional neural networks, deep reinforcement learning, and recurrent neural networks. The greatest advantage of DL is its efficiency and scalability. However, one its main challenges is that models require large amounts of data to learn from. Ideally, as technology progresses, learning efficiency will be higher, reducing the demand for high data volumes.

AI has greatly accelerated advances in biomedical research and healthcare. ML and DL are rapidly replacing manually generated workflows in computer vision and imaging and are now the predominant tools for pattern and object detection, segmentation, and classification. In biomedicine, they have been pivotal for disease diagnosis, prevention, personalised healthcare solutions, and clinical decision-making.

## 2.2 | Current uses of AI, ML and DL in parasitology

For this section, we conducted a systematic review of the existing body of work in Apicomplexan, Diplomonad and Kinetoplastid fields (methodology is shown in Figure 2).

**TABLE 1** Definitions and main concepts in AI, ML and DL.

| Name | Description |
|---|---|
| **Artificial intelligence** | AI defines intelligent machines capable of learning from past experiences, mimicking cognitive functions and human-like intelligence. There are many types of AI classifications including by functionalities (reactive machine AI, limited memory AI, theory of mind AI, and self-aware AI), and by capabilities (artificial narrow AI, general AI and super AI). |
| **Machine learning** | ML is a branch of AI that involves training algorithms to learn from and make decisions based on data. The learning process takes three steps: (1) Decision—based on input data, the algorithm produces a prediction about a pattern of the data. (2) Error function—this evaluates the prediction of the model. (3) Model optimisation—weights are adjusted to reduce the discrepancy between known examples and model estimates. ML can be classical or shallow (using only one hidden layer of nodes, e.g., linear regression or support vector machines), or deep (using multiple layers of nodes). |
| *Supervised machine learning* | This category of ML uses labelled datasets to train algorithms to predict outcomes or recognise patterns. Human experts generate annotations and algorithms learn the relationships between inputs and outputs. |
| *Unsupervised machine learning* | This category of ML uses algorithms to analyse and cluster unlabelled datasets. They discover hidden patterns or clusters without human intervention. One of their greater strengths is image and pattern recognition. |
| *Semi-supervised machine learning* | This category of ML uses a combination of labelled and unlabelled data to train predictive models. A small amount of labelled data is used to train an initial model. This is used to predict labels in larger amounts of unlabelled datasets. The resulting model is iteratively applied to labelled and pseudo-labelled data. |
| *Self-supervised machine learning* | This is an ML technique that generates implicit labels from unstructured data. It is particularly useful in computer vision. It makes tasks such as annotation by human experts, more efficient and cost-effective. |
| *Reinforcement learning* | This category of ML teaches systems to make decisions based on feedback from complex environments, to achieve optimal results. It mimics the process that humans undertake of 'trial and error'. When faced with a similar future environment, it will decide based on this experience. |
| **Deep learning** | DL is a branch of ML that uses artificial networks to learn from data. Algorithms are often trained on large datasets, often of labelled data. Neural networks are made up of layers of interconnected nodes, each of which is responsible for learning specific features of the data (e.g. edges, shapes, objects). Training of DL models can be done using supervised, unsupervised and reinforcement learning. Some of the most common types are described below. |
| *Convolutional neural networks* | They are often used for image recognition and processing, and excel at identifying objects, even when they are distorted or obscured. |
| *Deep reinforcement learning* | This combines reinforcement learning and deep learning. Valuable for tasks that require sequential decision-making, e.g. object localisation, image segmentation, registration. |
| *Recurrent neural networks* | They are often used for natural language processing and speech recognition. They excel at recognising the context of a sentence or phrase. |
| *Generative adversarial networks* | They use two neural networks to generate new data that resembles a training dataset. GANs have a generator and a discriminator. The former generates plausible data, while the latter distinguishes fake from real data. |

Artificial intelligence has been reported in parasitology since the early 2000s and has since experienced a significant increase across subfields (Figure 3A, B). Key achievements and publication trends are summarised in respective timelines (Figures 3A, B and 4). Light microscopy has historically been the gold standard for the diagnosis of most parasites of clinical relevance despite high time and expertise requirements. Delays and/or misdiagnosis are a significant detriment to public health; however, AI can exponentially increase the efficiency and accuracy of this process.

Correct diagnosis has become paramount in view of co-infections, and antimicrobial resistance. It is therefore not surprising that 90% of AI-based publications in the fields covered in this review use this technology for diagnosis. These studies focus on parasite detection, staging, and species classification from microscopy images (Figure 3C, Table 4). Below, we highlight key applications by parasite group (specific publication number and applications for each of the parasites covered are shown in Figure 4).

### 2.2.1 | Apicomplexans

Apicomplexans receive their name from the presence of a group of structures and organelles collectively known as the apical complex. This structure is thought to be key for host cell invasion. This section of our review focuses on *Plasmodium* spp., *Toxoplasma gondii, Babesia* spp., and *Cryptosporidium* spp. It does not include *Eimeria* sp., *Theileria* spp., or *Cyclospora* spp., as no relevant work was found at the time of publication.

**TABLE 2** Glossary of AI concepts reported in this review.

| Concept | Description |
|---|---|
| Accentuation edge via pixel value transformation | Modifying pixel values to emphasise edges in an image, making them more visually prominent. |
| AdaBoost | It is a type of ensemble boosting technique which iteratively trains multiple 'weak' models to create a strong classifier. It is heavily used in object detection applications, and can also be used as a supervisory layer for NNs, decision trees and SVMs. |
| Adaptive Moment Estimation (ADAM) optimiser | ADAM is a memory-efficient method that is highly effective for large datasets and complex models. It combines the strengths of Momentum (which smoothens out the trajectory of optimisations), and Root Mean Square Propagation (RMSprop—see term definition below). |
| AlexNet | Convolutional Neural Network that is considered revolutionary in image recognition. It won the ImageNet Large Scale Visual Recognition Challenge, demonstrating the power of DL for computer vision. |
| Association rules | It is a data mining technique that identifies patterns, connections or dependencies between different groups of items in data. |
| Autoencoder | Type of neural network that takes an input dataset and encodes it into a hidden layer. Then the encoded data is decoded and compared to the original input dataset. Depending on the degree of similarity between the two, the encoder might need to be tuned until a high degree of similarity between the two occurs. |
| Binarised greyscale hybrid algorithm with multiregion binarisation (BiGHAM) | A binarised greyscale hybrid algorithm combines multiple techniques to convert a greyscale image into B&W. For a multiregion approach, instead of using a single threshold, it uses multiple, each applied to specific regions of the image. |
| Bootstrap aggregating/bagging | Ensemble method that combines multiple models trained on bootstrapped datasets to improve model accuracy. The decisions of these trees are aggregated to produce a final prediction. |
| Canny edge detector | Edge detection operator that uses a multistage algorithm to detect a wide range of edges in images. |
| Capsular Network (CapsNet) | Type of neural network that overcomes limitations of conventional CNNs through the use of capsule neurons. These are better able to handle hierarchical structures. CapsNets can better recognise patterns in data, capture complex spatial hierarchies and improve generalisation. |
| Chaotic Cuckoo search | It is a metaheuristic optimisation algorithm that combines Cuckoo Search and chaotic mapping for greater performance. Cuckoo Search has the advantage of global search abilities, few parameters, and simplicity. Chaotic maps generate sequences with pseudo-random properties to tune parameters in the Cuckoo Search. |
| CiRA CORE | Central hub designed to bridge the gap between AI technology creation and applications. It facilitates the use of Robot Operating Systems. |
| Cluster assumption | A concept in semi-supervised learning that states that datapoints close to each other in the input space, likely belong to the same class, and form clusters. |
| Collateral motion saliency based models | Models used to identify and analyse motion that is not directly related to the object of interest, but rather, is generated as a result of its interactions with the surroundings. |
| Convolutional neural networks (CNNs) | Data is fed into the models to train them. They are used for image recognition, pattern recognition and computer vision. They use principles of linear algebra for pattern recognition within images. |
| Crowdsourcing | Method that enables leveraging the collective intelligence of large groups of people for tasks that aid in training AI models. |
| Cycle generative adversarial network (CycleGAN) | Deep learning model that allows image-to-image translation using unpaired datasets. It learns to map images between two different domains without requiring corresponding images of each. |

(Continues)

**TABLE 2** (Continued)

| Concept | Description |
|---|---|
| Decision trees | They seek to find the best split for a subset of data, and are commonly trained through a Classification and Regression Tree (CART) algorithm. Single decision trees can be prone to bias or overfitting, but when multiple decision trees form an ensemble in the random forest algorithm, they can predict more accurate results. |
| DeepAlign | Aligns sets of single particle images in the 3D space. |
| Deep reinforcement learning (DRL) | It combines reinforcement learning with deep neural networks to create algorithms that learn how to make decisions by interacting with the environment and receiving feedback (rewards or deterrents). The goal is to achieve a maximum reward. |
| Deep Transfer Learning (DTL) | A concept in machine learning that uses knowledge learned from one task to improve the performance of another related task. It is particularly useful when limited labelled data is available. |
| Deep cycle transfer learning (DCTL) | DTL that incorporates a cycle or feedback loop where the knowledge learned in the target task can be used to refine the source model. It results in reduced data needs, faster training, and improved generalisation. |
| Deep transfer graph convolutional networks (DTGCNs) | Neural networks that combine the strengths of GCNs and transfer learning. They learn node representations that capture local and global structural information within data. |
| DeepLeish | Deep learning based system for the detection of *Leishmania* parasites from microscopy datasets. |
| DenseNet121 | Pre-trained convolutional neural network architecture that is commonly used for image classification, especially as a basis for transfer learning. DenseNet have dense connectivity patterns, and each layer receives input from all previous layers. 121 is the number of total layers in the network. |
| Discriminative learning algorithms | They model the conditional probability distribution of output labels given the input features. Their goal is to define the threshold for judgements delineating different classes or categories. An example is logistic regression. In microscopy, it is mainly applied for image categorisation. |
| DotFinder | Techniques in DL that enable the identification and location of specific 'dots' within an imaging dataset. |
| DropConnect | It helps regularise fully connected layers within neural networks. It works by setting a randomly selected number of weights within the network to zero. |
| EasyScanGO | Device that uses machine learning-based image analysis for automated parasite detection. |
| EfficientNetB2 | Sophisticated CNN model that represents an advancement in mobile-friendly computer vision architectures. |
| Faster recurrent neural networks (Faster R-CNN) | Strategies implemented to optimise other existing architectures to accelerate their performance. Methods include the development of Fast GRNN, FS-RNN, using parallel computing and specialised hardware. |
| Feedforward neural network | Data is fed into the models to train them. They are comprised of sigmoid neurons and they are fundamental for fields such as computer vision, natural language processing, among others. |
| Federated learning frameworks | Tools that facilitate the training of models across multiple, decentralised devices or servers without needing data to be centralised. They allow collaboration while preserving data privacy and security. |
| FiRCoN | DL-based algorithm that automatically quantifies intracellular parasite burden from fluorescence microscopy images. It uses FCRNs to generate density maps. |
| Fine-tuned optimisers | Specialised algorithms that improve the training process of pre-trained models. They incorporate strategies that ensure efficient and effective learning during the fine-tuning phase. They iteratively adjust the model's parameters to minimise loss of function. |

(Continues)

**TABLE 2** (Continued)

| Concept | Description |
|---|---|
| Fully convolutional neural network (FCNN) | Type of CNN that uses only convolutional layers instead of fully connected layers, and can accept input of variable sizes. It is well suited for semantic segmentation, where each pixel in an image is assigned a class label. It enables end-to-end learning. |
| Fully convolutional regression network (FCRN) | FCNN specifically designed for regression tasks, with the goal of predicting continuous values as opposed to discrete categories. |
| Fuzzy cycle generative adversarial networks (FCGAN) | They are GANs that incorporate fuzzy logic to enhance their performance. Fuzzy logic enables the representation of concepts and relationships using degrees of membership instead of binary values. It is better suited for handling uncertainty and imprecision in data. |
| Gaussian mixture models (GMMs) | Used in unsupervised learning; this probabilistic model determines the probability each data point has of belonging to a given cluster. |
| Gaussian discriminant analysis | Used to solve multiclass classification problems. Assumes that the data in each class follows a Gaussian distribution. It then uses Bayes' theorem to compute the probability that a new data point belongs to each class, and chooses the one with the highest probability as the predicted class. It can handle data with arbitrary covariance matrices for each class. |
| Generative learning algorithms | They learn the patterns in a given dataset and use it to create new data with similar characteristics. They include Gaussian discriminant analysis and Naïve Bayes. |
| Genetic programming | Evolutionary algorithm that operates on a population of programs and applies genetic operators selection according to a predefined fitness measure, mutation and crossover. It produces a new generation of programs that are on average more fit than members of the previous generation. |
| Geometric feature spectrum ExtremeNet | It performs bottom-up object detection by grouping extreme and centre points with an anchor-less framework. |
| Gradient-weighted Class Activation Mapping (Grad-CAM) | DL method to visualise which parts of an input image are most important for a particular class prediction. It generates a heatmap that highlights the relevant regions. |
| Gradient boosting | This is a type of ensemble supervised ML algorithm that combines multiple 'weak' learners to create a strong learner. Each predictor is trained using the residual errors of the predecessors as labels. |
| Hierarchical clustering | Unsupervised learning method that builds clusters by measuring dissimilarities between data. There are two main types of hierarchical clustering: agglomerative (bottom-up) and divisive (top-down). The former starts with each observation in its own cluster and then merges pairs of clusters going up the hierarchy. The latter starts with all observations in a single cluster and then splits them as it moves down the hierarchy. |
| Higher order local autocorrelation (HLAC) | Feature extraction technique used in image processing that considers relationships between pixels at multiple distances. This allows investigating more complex local patterns than traditional autocorrelation. |
| Histogram-oriented region | Feature descriptor technique used in computer vision and image processing for object detection. Its focus is on the shape of the object, counting the occurrences of gradient orientation in each local region. |
| Hybrid deep learning | It combines multiple deep learning architectures, or integrates DL and ML techniques, to leverage the strength of all approaches. |
| Image foresting transform | The transform makes a graph of the pixels in an image. The connections between points are the 'cost' of the path portrayed. Trees are made by connecting the pixels that have the same or close cost for applying the operator decided upon. |
| ImageNet | A large scale, hierarchical database of annotated images used for visual object recognition research. It is a key source for training and testing DL models in computer vision. |

(Continues)

**TABLE 2** (Continued)

| Concept | Description |
|---|---|
| iMAGING | Automated system for malaria diagnosis that uses AI tools and a low-cost robotised microscope. |
| Inception-ResNet-V2 | Deep convolutional network architecture that combines the inception module with residual connections. V2 contains multiple inception modules interleaved with residual connections. It is trained in more than one million images from the ImageNet database. It can classify images into 1000 object categories. |
| Invariant-moments adaptive network based fuzzy interference systems (ANFIS) | Invariant moments are mathematical features derived from an image that remain unchanged under transformations such as rotation, scaling and translation. Adaptive network based fuzzy interference systems (ANFIS) combines feature extraction using invariant moments, with fuzzy logic for robust classification and pattern recognition. It can handle uncertainty and non-linear relationships in data. |
| Inverted residual blocks | Building blocks used in convolutional neural networks. They are particularly 'lightweight' architectures used for mobile and embedded devices. |
| KERAS ResNet50 | KERAS is an open source library that provides Python interface for artificial neural networks. It simplifies the process of deploying NNs. It is written in Python and can be used on top of backends like TensorFlow, JAX and PyTorch. ResNet50 is a residual network with 50 layers, that was developed for visual recognition tasks. |
| KNIME Analytics platform | Konstanz Information Miner (KNIME) is an open-source data analytics platform that enables users to build data analysis workflows with little coding knowledge. |
| LeishFuNet | DL model designed for the automated detection of *Leishmania* spp. amastigotes from microscopic images. |
| Linear discriminant analysis | Used to solve multiclass classification problems. It identifies a linear combination of features that characterises two or more classes of objects or events. Often used to enhance other learning classification algorithms such as decision trees, random forest, or support vector machines. |
| Linear regression | In supervised ML, it learns the relationship between a set of features and a label, mapping them to the most optimised linear functions. These can then be used to predict new datasets. |
| Logistic regression | In supervised ML, logistic regression helps perform binary classification tasks by predicting the probability of an outcome of an instance belonging to a given class or not. |
| Long short term memory (LSTM) convolutional autoencoders | They can capture long dependencies in sequential data, making them ideal for time series forecasting. LSTMs have a memory cell that holds information over extended periods. This helps address the issue of learning long-term dependencies. |
| MCellNet | Neural network optimised to distinguish between microorganisms such as Cryptosporidium, Giardia, microplastics, and water pollutants from brightfield images. |
| MIA-QSAR | Stands for multivariate image analysis-quantitative structure-activity relationship methods which is able to relate chemical structures with biological activities. |
| Microscopic-macroscopic associated object pulling | Strategy that uses knowledge transfer from macroscopic domains to improve recognition of microscopic objects. The association of one with the other aids the recognition process. |
| Mimicry embedding | Technique that prepares new datasets for use in ML/DL by transforming them to resemble a known, verified dataset. Weights on the verified dataset can be transferred and fine-tuned on the new dataset. |
| MobileNetV2 | Lightweight and efficient CNN architecture designed for mobile and embedded vision applications. It uses inverted residual blocks and linear bottlenecks, which results in higher accuracy and speed, while maintaining low computational costs. |
| Multiclass support vector machines | SVMs are efficient in binary classification tasks. Multiclass SVMs are able to distinguish between more than two classes. |

(Continues)

**TABLE 2** (Continued)

| Concept | Description |
|---|---|
| Neural networks (NNs) | Neural networks mimic the interconnectivity of neurons in the human brain through layers of nodes (or artificial neurons). Each node has an input layer, one or more hidden layers, and an output layer. If an output value exceeds a given threshold, it 'fires' or activates the node, passing data to the next layer in the network. The most common types of neural networks are Feedforward Neural Networks (FNNs), Convolutional Neural Networks (CNN) and Recurrent Neural Networks (RNN). |
| Neuro-fuzzy systems | Hybrid systems that combine the strengths of neural networks and fuzzy logic. This yields it the strengths of both including learning and adaptation, and the ability to handle uncertainty and vagueness. These systems are able to generalise knowledge, and make decisions in situations with incomplete or imprecise data. |
| Probabilistic neural networks | It is a type of architecture designed for classification tasks by the use of Bayesian statistics and probability theory. It tackles classification and pattern recognition problems through a statistical approach. |
| Radial basis function (RBF) | Function whose value depends on the distance (generally Euclidean) to a centre in the input space. |
| Random forest | In supervised ML, random forest algorithm is used for both classification and regression. The forest refers to a collection of uncorrelated decision trees, which are merged together to reduce variance and create more accurate predictions. |
| Recurrent neural networks | They use feedback loops and are most valuable to make predictions about future outcomes. |
| ResNet2SVM | Allows combining the features of deep residual network with SVM. |
| ResNet18 | Popular CNN made of 18 layers, known for its deep learning capabilities in image recognition. |
| RetinaNet | One-stage object detection model that uses a focal loss to address class imbalance. It is simple, achieves high accuracy, and is fast at inference. |
| RMSProp | Root Mean Square Propagation is an adaptive learning rate optimisation algorithm used in training DL models. It helps stabilise training, especially in situations with noisy, sparse, or non-stationary objectives. |
| RT-DETR | Real time detection transformer (RT-DETR) is an object detection algorithm of high accuracy and speed. The transformer architecture allows tit to process sequential data and capture long-range dependencies. It can do object detection in real time, and can be adapted to different speed requirements without retraining. |
| Stochastic gradient descent (SGD) | Iterative optimisation algorithm used to find the minimum of a function in ML models. |
| Siamese-based deep metric | It combines Siamese NNs with deep metric learning. Siamese networks are characterised by two or more identical subnetworks that share the same architecture, weights and parameters. They can compare two or more input vectors and determine their similarity. |
| Supervised multinomial logistic regression | Statistical classification method used to predict the categorical outcome with more than two possible values. It allows for multiple, unordered classes in the predicted variable. |
| Support vector machines (SVM) | In ML, SVMs are used for data classification and regression. They construct a hyperplane where the distance between two classes of data points is at its maximum. This decision boundary separates the classes of data points on either side of the plane. |
| Transfer learning | Technique where a model trained on one task is reused as a starting point for a different but related task. |
| U-Net | It stems from the fully convolutional network and is primarily used for image segmentation tasks. It is designed as an encoder-decoder structure with skip connections. |

(Continues)

**TABLE 2** (Continued)

| Concept | Description |
|---|---|
| Vector database-based image retrieval systems | They leverage the power of vector databases to efficiently store, manage and retrieve images based on their content. |
| YOLO | You only look once—refers to a real time detection algorithm in computer vision. It is known for its speed and efficacy, allowing it t process a large number of frames per second. The YOLO family includes multiple versions, each with improvements in speed, accuracy and functionality. |
| YOLO v4 tiny | Compressed version of YOLO v4, designed to train on machines that have less computing power. |
| YOLO v5 | Versatile and powerful object detection model that is easy to use and deploy. It has been useful in various computer vision tasks, including object detection, instance segmentation and image classification. It is implemented in the PyTorch framework. |
| YOLO v5s | It is a version of YOLO v5 that balances speed and accuracy. |
| YOLO v5x | It is the largest and most complex model of the YOLO v5 family, achieving the highest accuracy. |
| YOLO v7 | A later version of the YOLO family that outperforms YOLO v5 in speed and accuracy. It can struggle with small object detection and scale variation. It is also computationally intensive. |

### Plasmodium *spp. and malaria*

The bulk of the AI-powered microscopy-based studies reported for malaria research, account for over 75% of the work reported in this review. In the vertebrate host, three main stages take place: the pre-erythrocytic stage in the liver, the erythrocytic stage (in red blood cells (RBCs)), and gametocytogenesis—with gametocyte maturation occurring in the bone marrow. An overview is shown in Figure 5A, left panel.

*Overview of the* Plasmodium *life cycle.* ***Plasmodium*** spp. have a two-host life cycle: one in the mosquito vector and one in the vertebrate host. Following an infected mosquito bite, two key types of cells are invaded in the vertebrate host: hepatocytes and red blood cells (RBCs). In hepatocytes, *Plasmodium* hijacks host organelles and nutrients to fuel what is thought to be one of the fastest replication rates in eukaryote biology.[31,32] Once this stage is complete, parasites are released to the bloodstream where they infect RBCs. Within infected RBCs (iRBCs), *Plasmodium* undergoes multiple rounds of asexual replication (passing through different stages of maturation known as ring, trophozoite and schizont), which culminates in the release of multiple offspring (called merozoites). These offspring invade new RBCs and repeat the cycle (Figure 5A, left panel). A small percentage of these offspring will become sexual forms or gametocytes, a terminal stage which is nonetheless necessary for transmission to the mosquito.

*Diagnosis in RBCs: identification, staging, species identification and classification.* Malaria is highly endemic across Latin America, Sub-Saharan Africa, and Southeast Asia, with five species being responsible for clinical cases: *P. falciparum*,[33–35] *P. vivax*,[36,37] *P. malariae, P. ovale*,[38] and *P. knowlesi*.[39–41] Their morphology in peripheral blood is very similar, making microscopy-based diagnosis a very complex task (Figure 5A, right panel). Albeit challenging, species identification is possible due to subtle yet unique morphological and developmental landmarks. Correct classification between species is crucial because it determines the form of treatment and triage to be followed. Each species results in different levels of lethality (with *P. falciparum* being the most lethal), and some of them generate latent forms (hypnozoites) (*P. vivax* and *P. ovale*) that remain dormant in the liver and eventually re-emerge. Moreover, *Plasmodium* spp. have several developmental stages during their asexual life cycle in the host RBCs: rings, trophozoites, schizonts, and gametocytes. Depending on the ratio of these stages in circulation, features such as disease progression and treatment efficiency can be assessed.

We identified over 130 publications that have implemented or designed tools for semi-automated or fully AI-powered malaria diagnostics, focusing on identifying and classifying the blood stages, and the parasite species. We refer readers to a systematic review covering advances until 2015,[42] as well as current reviews.[43–47] Key historical achievements go from the early development of tools like MalariaCount,[48] MIA-QSAR,[49] and Malaria Screener[50] to the development of more sophisticated platforms like EasyScanGO[51,52] and iMAGING,[53] the adoption of genetic programming[54] and deep transfer learning.[55–59] Additional to state-of-the-art ML and DL approaches, a high priority has also been given over the last decades

**TABLE 3** Common pitfalls of ML and DL as relevant to imaging and parasitology.

| Pitfall | Description |
|---|---|
| Overfitting | It occurs when a model learns too much from the training data, including details that don't matter. This makes it difficult to generalise and apply to new data. |
| Underfitting | An underfitting model fails to capture the underlying patterns in the data, resulting in poor performance in training and unseen data. |
| Ignoring data quality | ML and DL algorithms learn from the data they are trained on. If the data is flawed, the models can inherit those flaws. Poor data quality includes incomplete or inaccurate data, outdated data, redundant data. Incomplete datasets, inaccurate annotation, among others. |
| Wrong algorithm | Choosing the wrong algorithm for a given data will likely lead to inaccurate predictions. |
| Data leakage | This occurs when data from outside the training dataset is used to create the model, but this future data will be unavailable when the model is used for prediction. This leads to highly optimistic performance estimates, and poor generalisation and use on new, unseen data and real world applications. |
| Failing to tune hyperparameters | Tuning parameters is critical for optimal model performance. Examples of hyperparameters include learning rate, batch size, and number of epochs. Examples of tuning methods are grid search, random search and Bayesian optimisation. |
| Unnecessary model complexity | Using a model that is more complex than it requires to address a certain question can result in overfitting, increased computational cost, and reduced interpretability. Ways to address this issue include choosing a model with fewer parameters and layers, and stopping the training process earlier. |
| Out-of-distribution data | This refers to data that a model hasn't seen during training, which can lead to unreliable predictions. OOD is key to ensuring the reliability of ML systems. |
| Irrelevant feature selection | This involves identifying and removing features that do not contribute to the predictive accuracy or classification tasks of a model. Reducing the number of features can improve the accuracy and efficiency. Conversely, irrelevant features can lead to overfitting, reduced model performance, and increased complexity. |
| Inadequate data splitting | Inadequate splitting of the data can lead to models that are too specific for the training data, bias, and failure to capture important patterns. They don't generalise well to new data. |
| Insufficient model evaluation | Adequate model evaluation includes using appropriate evaluation metrics, doing cross-validation, and monitoring the model's performance over time. This can result in inadequate predictions and poor generalisation. |
| Imbalanced data | This refers to when the classes within a dataset are not represented equally. If one class has a significantly higher proportion of instances than the other, this can result in biased models. |
| Noisy data | This results from data coming from multiple sources with varying quality, data processing issues, and data collection errors. |
| Bias and fairness | This can be mitigated by using fair algorithms, addressing biases in the training data and implementing bias detection and mitigation methods. |
| Ethics | One must ensure that algorithms are used responsibly, promoting transparency, accountability and social responsibility. Areas of concern include algorithmic bias, fairness, privacy, and accountability. This is of particular importance in the public health aspects covered in this review. |
| Data privacy | Personal information should be protected from unauthorised access and misuse- in this case, patient identifiers. We should abide by data protection regulations, take relevant data security measures, and follow adequate data provenance. |
| Legal considerations | These can include data privacy, intellectual property, bias and discrimination, malpractice and negligence among others. In this work, for instance, we should think about the potential harm caused by misdiagnosis and who the liability and accountability falls on. |

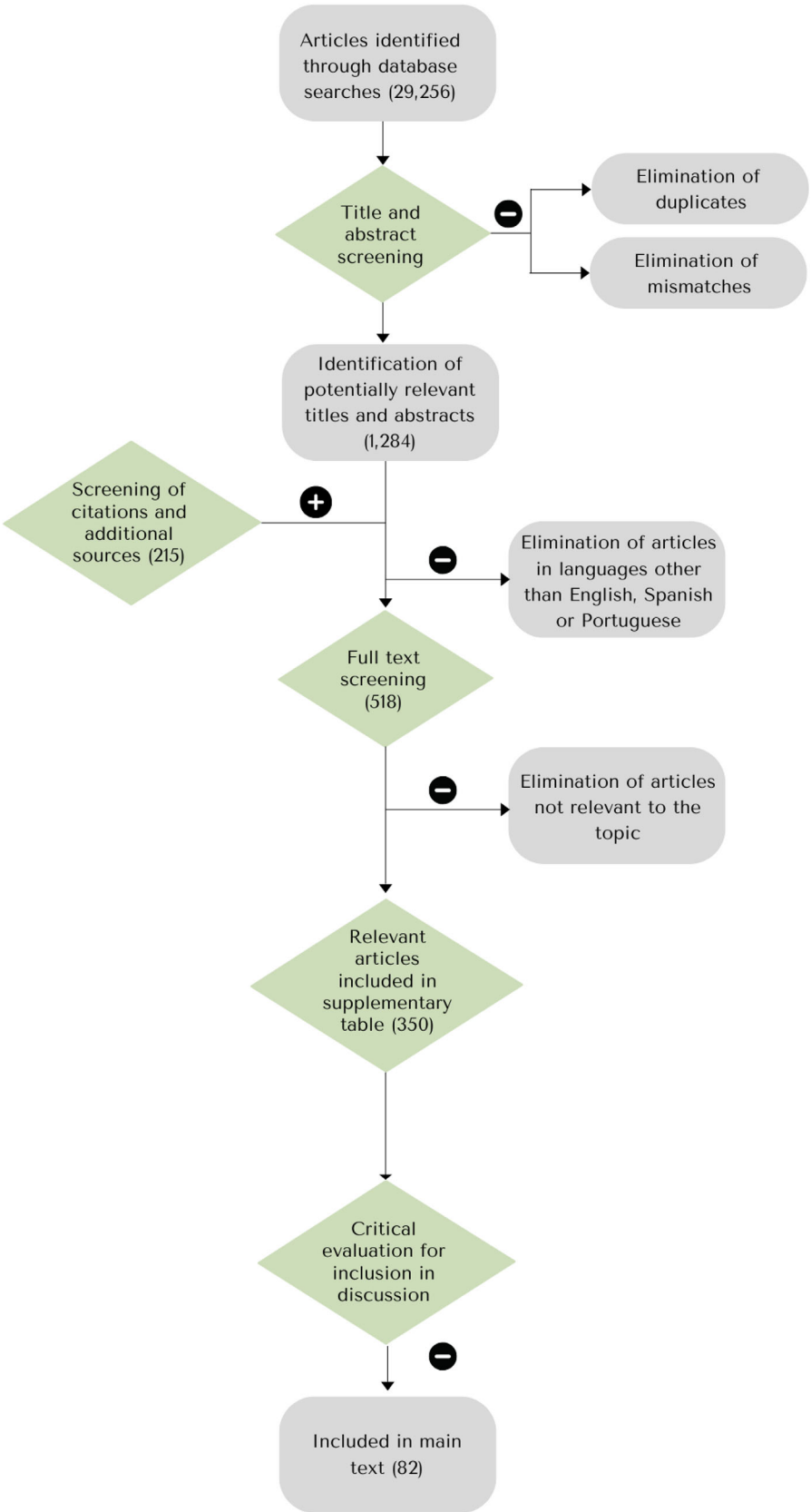


**FIGURE 2** Methodology used for systematic review. We describe the workflow we followed that led to identification and inclusion of studies addressed in this work. Figure was generated with Canva (canva.com).

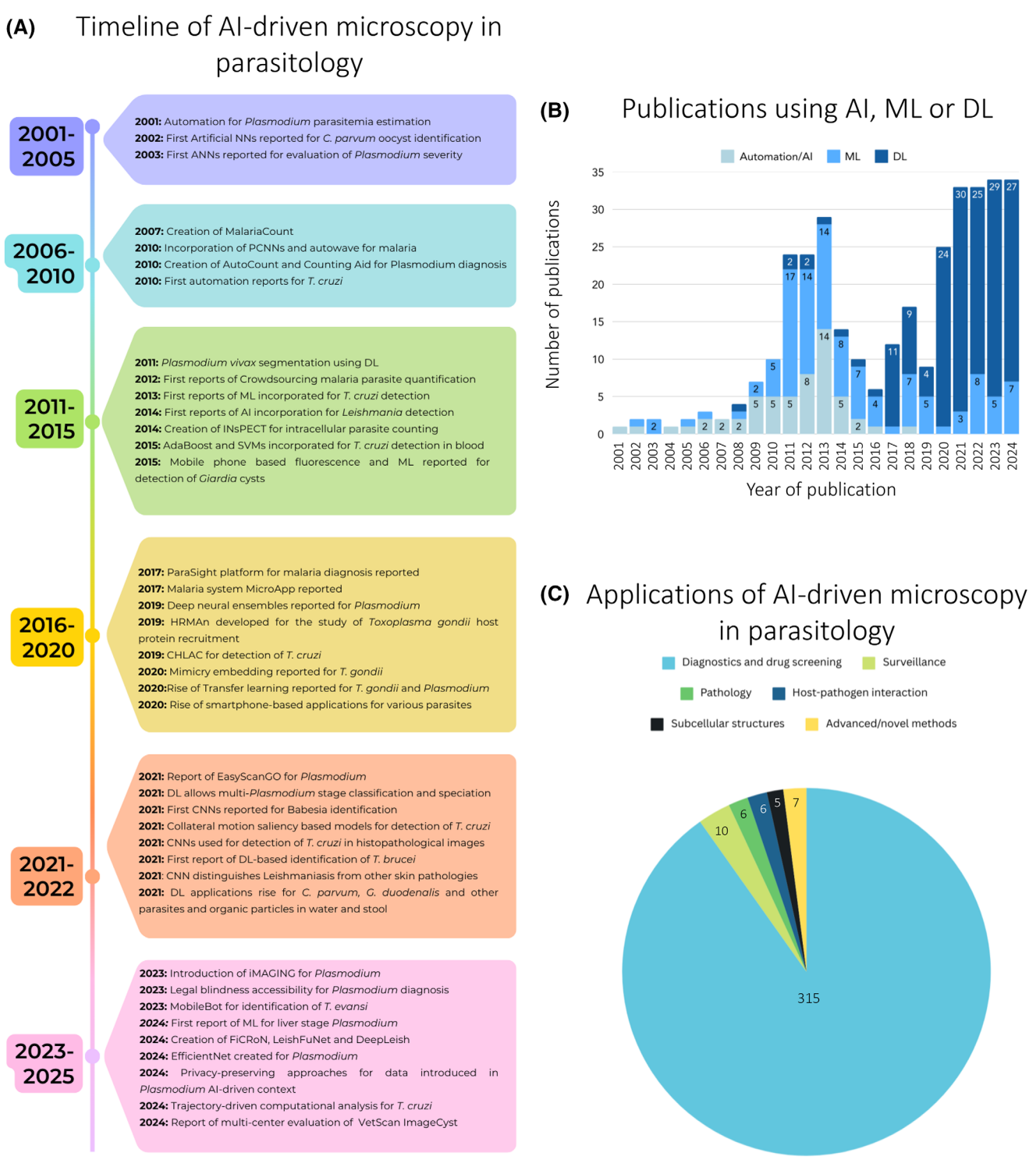


**FIGURE 3** Timeline of key events and publications using AI-powered microscopy. (A) Timeline showing critical developments in AI-powered microscopy for parasitology, spanning from 2001 to January 2025. (B) Number of publications per year across apicomplexan, diplomonad and kinetoplastid fields, reporting the use of AI (light blue), ML (blue) or DL (dark blue). (C) Main uses of AI-powered microscopy in parasitology. Diagnostics and drug screening (shown in light blue) represents 90.2% of the work using AI-powered microscopy, followed by water surveillance (light green, 3%), pathology (dark green, 1.7%), host–pathogen interactions—referring mostly to basic science research using cells in vitro (dark blue, 1.7%), subcellular structures (black, 1.4%) and advanced or novel methods (yellow, 2%). Figure was generated with Canva (canva.com).

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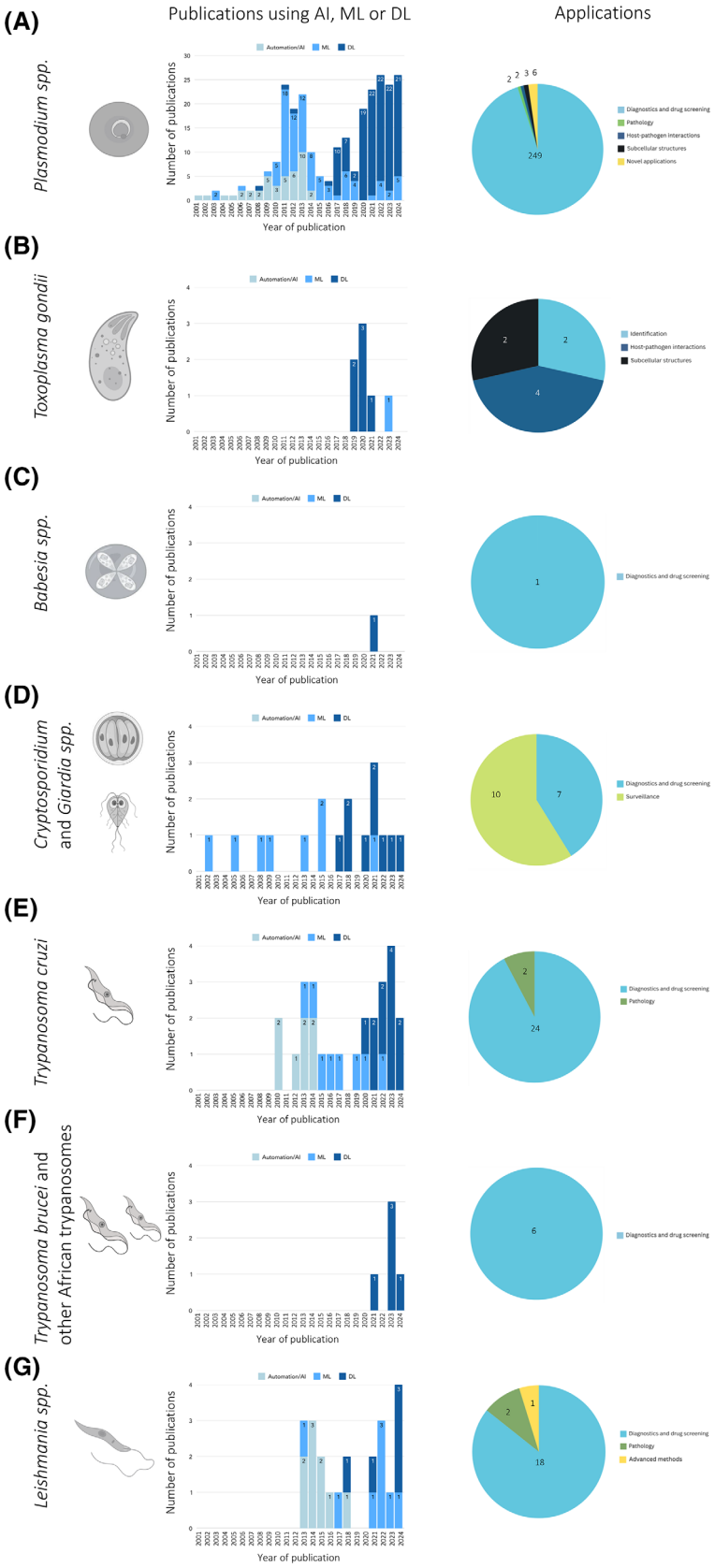



**FIGURE 4** Timeline of publications and applications using AI-powered microscopy by parasite. The first column shows schematics of relevant parasites. (A) *Plasmodium*, (B) *Toxoplasma gondii*, (C) *Babesia*, (D) *Cryptosporidium* spp. and *Giardia* spp., (E) *Trypanosoma cruzi*, (F) *T. brucei* and other African trypanosomes, and (G) *Leishmania* spp. The second column shows the number of publications by year using AI or automation identified as AI in search algorithms (light blue), ML (blue) and DL (dark blue). The third column shows main uses of AI-powered microscopy in parasitology by parasite (A–G). Colour codes are: Diagnostics and drug screening (light blue), surveillance (light green), pathology (dark green), host–pathogen interactions (dark blue), subcellular structures (black) and advanced or novel methods (yellow). Figure was generated with Canva (canva.com) and parasite diagrams were generated with BioRender.com.

to mobile technologies[60–65] due to the need to address malaria diagnosis in field and remote locations.

Over 66 studies using DL and ML for microscopy-based malaria diagnosis have been published in the last two years alone. Due to the volume of published work, and that reviews exist covering achievements spanning several decades prior to 2022, we focus here on 3 contributions developed in the last year (in the field of parasitology) achieving higher detection and classification accuracy than several preceding technologies.

- **EfficientNet-B2**:[66] This is a convolutional neural network (CNN) model mostly used for image classification, ideally suited for problems that require few parameters and have minimal processing resources. Key attributes of this tool, compared to many of its predecessors is its high accuracy and efficiency in iRBC detection, while avoiding overfitting (a common problem faced using earlier algorithms).
- **Real-time detection transformer (RT-DETR) object detection algorithm**:[67] This algorithm was designed to identify 5 different haematozoan parasites, including 4 *Plasmodium* spp. divided into 3 classes. RT-DETR eliminates the need for computationally expensive segmentation, enabling its use with smartphones with a microscope adapter.
- **Transfer learning**:[45,55–59] Transfer learning helps address a common hindrance in parasitology, which is the lack of large, balanced well-verified datasets. In transfer learning, knowledge gained through one task is transferred onto a fully or partially identical untrained network, and used to improve model performance on a related task or dataset.[68] This results in shorter training time and is considered more efficient than random weights initialisation strategies.[69] This ML method has been increasingly adopted for *Plasmodium* detection and diagnosis, with multiple studies using versatile transfer learning approaches.

*Liver stages.* After the bite of an infected *Anopheles* mosquito, *Plasmodium* sporozoites travel from the skin to the blood vasculature, and display tropism to the host liver. In hepatocytes, parasites form a parasitophorous vacuole membrane in which they undergo fast asexual replication, before progeny merozoites are released to the bloodstream.[70,71]

To our knowledge, only one study has used ML-powered microscopy to focus on the liver stage. Otesteanu et al.[72] used CNNs to extract physical parameters characterising parasite development during the liver stage of infection, and to predict the transition of *Plasmodium berghei*-infected liver cells to the formation of freely moving merozoites. Out of 17 extracted features, four of the most important for characterising development were volume, convex envelope, and mean and maximum signal intensity. Overall, automated cell segmentation and tracking allowed identification of developing parasites and achieved high prediction accuracy on which parasites were able to progress to a stage where merozoites freely navigated within host cells.

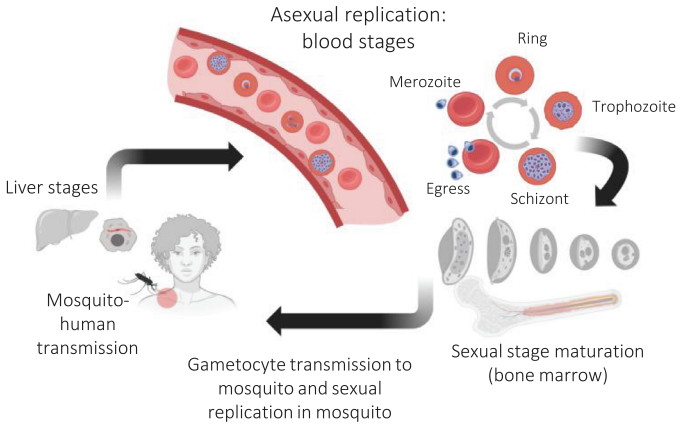
*Gametocyte stages.* Gametocytes are the sexual stages of *Plasmodium* spp., divided into male and female gametocytes, which can be readily taken up by *Anopheles* mosquitoes. They are central to malaria transmission[73] and have been the focus of transmission-blocking antimalarial treatment development.

While several diagnosis pipelines include gametocytes in their classification output,[74–76] to our knowledge, only one study has fully focused on the use of AI-powered microscopy for gametocyte investigation. Tsebriy et al.[77] developed 'Phenotype Image Data and Digital Learning Innovation' (PhIDDLI), an ML analysis pipeline to characterise the phenotype of male gametocytes. Many transmission-blocking molecules act by rendering the gametocytes sterile, or by interfering with gametogenesis. Based on gametocyte morphology, the pipeline helped identify which cells had progressed into male gametogenesis, and which ones had arrested before exflagellation. Moreover, it also helped reveal gametocyte clusters that coincide with groups of drugs that act through different mechanisms of action to arrest development. Ultimately PHIDDLI helped identify *Plasmodium* kinases essential for gametogenesis, given the drastic effect of kinase inhibitors tested.

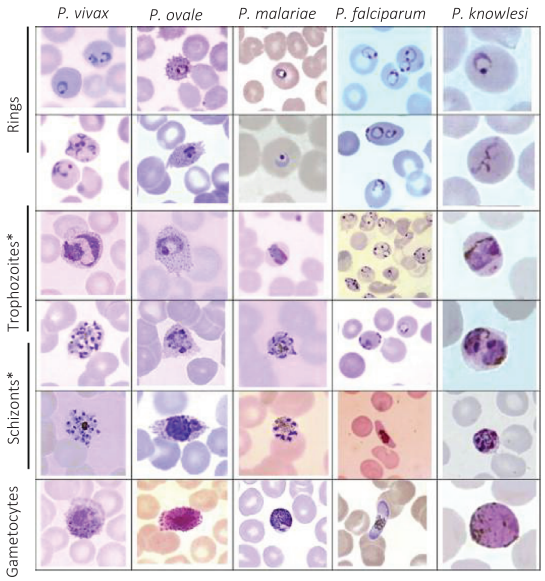
*Applications to pathology.* Malaria-associated pathology includes severe malarial anaemia, placental malaria, cerebral malaria, acute respiratory distress, and metabolic acidosis, among others. *Plasmodium falciparum*-related cerebral malaria is associated with sequestration of infected RBCs to the blood vasculature in the brain. However, cerebral malaria has a complex clinical presentation and identifying mechanisms governing pathology has been a challenging task. Two studies have reported the use of AI-powered imaging in this context.

Kessler et al.[78] combined MRI-based imaging of the brain, funduscopic examination of the eyes, parasite transcript profiles, and laboratory blood profiles of clinical paediatric patients to develop ML models of malarial retinopathy and brain swelling. ML models were based on random forests to discriminate patient groups with various clinical classifications. Key findings include that parasite biomass, low platelet levels, and specific PfEMP1 variants (EPCR-binding) are important for cerebral malaria pathogenesis. In 2021,[79] the same group expanded their original

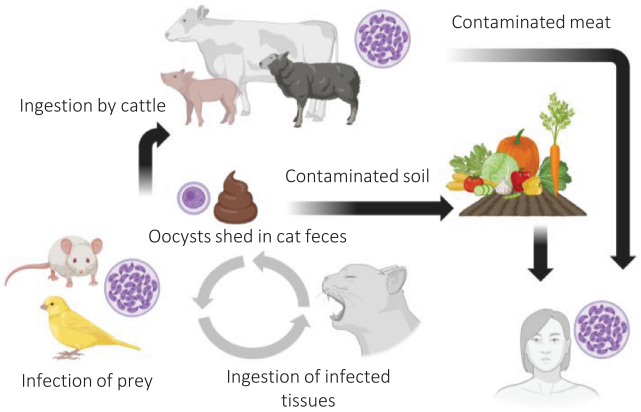
**(A)** *Plasmodium:* multiple applications




*Plasmodium* staging and species classification

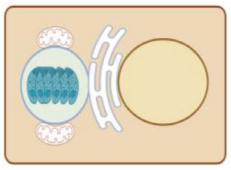



**(B)** *Toxoplasma gondii:* multiple applications

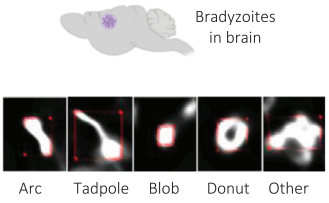



HRMAn

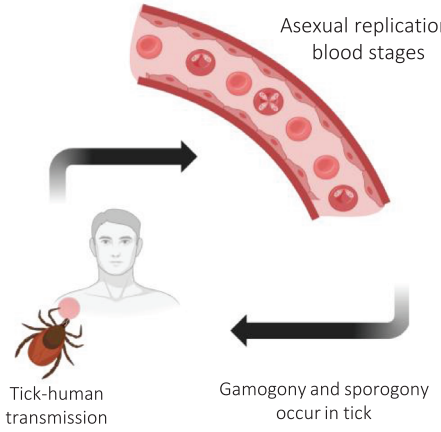
Intracellular host-parasite interactions



*T. gondii* mitochondrial morphology

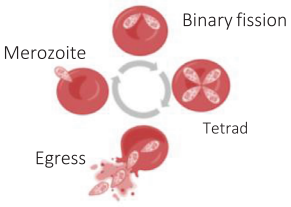


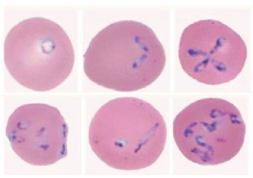

**(C)** *Babesia* identification




Differential classification

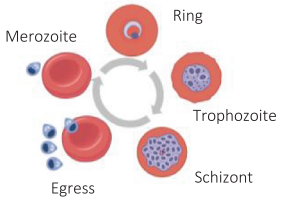
*Babesia spp.*

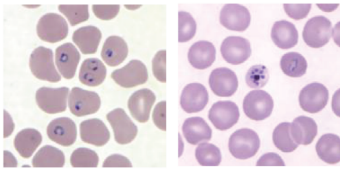


*vs.*

*Plasmodium spp.*



work to investigate whether similar factors are linked to cerebral malaria and brain swelling across human populations (comparing cohorts in India and Malawi), and across ages (adults versus children). They found that their ML models trained in African children performed well in classifying cerebral malaria in Indian children, but less well in classifying adults. Overall, higher parasite biomass and Group A-EPCR binding variants were common features in children and adult cerebral malaria cases.

*Infected mosquito identification.* Vertebrate-to-insect vector transmission occurs when *Anopheles* female mosquitoes take up a bloodmeal from an infected human, ingesting gametocytes which then undergo sexual replication in the mosquito's midgut.[80] The proportion of malaria-infected versus non-infected mosquitoes is an entomological metric that helps investigate the intensity of transmission, the impact of malaria control measures. This calculation is also key in the process of surveillance towards malaria elimination. Conventionally, the estimation of infected mosquito rates is done by dissecting salivary glands and quantifying how many have sporozoites. This is a laborious process that requires expertise.

In their work, Esperança et al.[81] evaluated near-infrared spectroscopy (NIRS) to identify mosquitoes infected with the rodent parasite *P. berghei*, in a laboratory setting. They implemented a ML approach to classify NIR spectra to differentiate between infectious and non-infectious mosquitoes and achieved a relatively high accuracy (72%). The study discloses, however, that the exact parameters that allow differentiation between infected and uninfected mosquitoes are unknown. The authors also emphasise the need to test this method in natural settings as opposed to lab-controlled conditions.

*Subcellular resolution.* The visualisation and understanding of subcellular structures are key to the study of microbiology. The field of *Plasmodium* has implemented AI-powered methods in two different fields of microscopy: cryo-electron microscopy (cryo-EM) and atomic force microscopy (AFM).

Single particle analysis for cryo-EM is a valuable tool to determine 3D structure of macromolecules, and molecular interactions. A hindrance for image analysis of 3D maps, however, is the highly error-prone 3D alignment step. Jimenez-Moreno et al. created DeepAlign,[82] a CNN-based solution for alignment. They compared the performance

**FIGURE 5** Overview of *Plasmodium, Toxoplasma gondii*, and *Babesia* life cycles and main applications of AI-powered microscopy. (A) *Plasmodium*. Left panel: overview of the life cycle. *Plasmodium* is transmitted to humans through the bite of an infected female *Anopheles* mosquito. Injected sporozoites move from the bite site via the bloodstream and show preferential tropism to the liver. Here, they invade hepatocytes, where they form a parasitophorous vacuole membrane where they undergo fast asexual replication giving rise to thousands of merozoites. Merozoites egress the liver in merosomes, through which they reach the blood vasculature. Upon egress from merosomes, merozoites invade red blood cells (RBCs). In RBCs, they undergo multiple rounds of asexual replication. During the erythrocytic cycle, merozoites transform into rings, which transform into trophozoites, and finally become schizonts. Schizonts undergo segmentation to undergo daughter merozoites which egress the infected RBC and invade new RBCs. Some parasites commit to gametocytogenesis. Gametocytes are the sexual forms of the parasite, and undergo maturation in the host bone marrow. Mature gametocytes egress the bone marrow and return to the bloodstream. They can be taken up by mosquitoes upon a bloodmeal. In the mosquito midgut, they undergo sexual replication, resulting in the production of sporozoites. AI-powered microscopy has been used to address multiple questions in this process. Right panel: The most widely used application of AI in *Plasmodium* imaging is diagnosis, both for parasite identification, staging and species classification. The panel shows different developmental stages of the five *Plasmodium* species that can infect humans. Image reproduced with permission from Garcia LS *Malaria Clin Lab Med* 30. 93–129, 2010. *P. knowlesi* column courtesy of CDC). (B) *Toxoplasma gondii*. Left panel: overview of the life cycle. *T. gondii* is transmitted to humans via the fecal-oral route. Sexual replication takes place in felines including cats. Unsporulated oocysts are shed in the cat's feces, which sporulate in the environment and become infective. Intermediate hosts such as birds and rodents can become infected after ingesting contaminated soil, water, or plants. Oocysts transform into tachyzoites after ingestion. Cats can become re-infected after ingesting intermediate hosts. Humans can become infected through the ingestion of undercooked meat of infected animals, infected water or soil, or direct exposure to cat feces. Tachyzoites preferentially invade muscle tissue. In neural tissue, tachyzoites can become tissue cyst bradyzoites. AI-powered microscopy has been used to address multiple questions in this process.Right panel: Examples of applications of AI-powered microscopy include the development of HRMAn to study intracellular host–parasite interactions and the study of mitochondria morphology in bradyzoites. Image shows the most commonly found mitochondrial morphologies: arcs, tadpoles, blobs, donuts, and other. This panel has been modified and reproduced with permission from Place et al. (C) *Babesia* spp. Left panel: *Babesia* spp. usually involve several hosts including rodents. Humans become infected through the bite of an infected tick of the genus Ixodes. Sporozoites are introduced through an infectious bite, and invade RBCs. Here they undergo multiple cycles of asexual replication. During this process *Babesia* spp. undergo morphological modifications including rings, Maltese cross, Accole, coccoid forms and amoeboid forms. Right panel: AI-powered microscopy has been used for parasite identification and differentiation of *Babesia* spp. from *Plasmodium* spp. Images adapted from Herwaldt et al. (2004), CDC (https://doi.org/10.3201/eid1004.030377) and Garcia (2010), Clin Lab Med (https://doi.org/10.1016/j.cll.2009.10.001) (both reproduced with permission). Figure schematics were generated with BioRender.com.

of DeepAlign with other methods including Xmipp Highres,[83,84] Relion,[85] and cryoSPARC.[86] Among the structures used for validation, was *Plasmodium falciparum* 80S ribosome, which helped establish DeepAlign as a robust method for cryo-EM alignment.

*Plasmodium falciparum* releases extracellular vesicles (EVs) during the erythrocytic stage of infection, to modulate host responses, for parasite-parasite communication,[87] and RBC priming and erythropoiesis.[88] In 2022, Abou Karam et al.[89] combined hybrid modalities to define whether specific subpopulations of EVs exist: using a Laurdan staining assay, combined with AFM and ML methods, they showed that there are two distinct subpopulations of EVs, which display different biophysical properties (i.e. membrane rigidity), protein content, and fusion capacity. This finding opens new avenues for research on how different EV subpopulations may target different cell types of pathways.

*Novel applications.* Beyond direct research- or clinical-specific applications, AI-powered microscopy has been used in various novel and creative ways within the *Plasmodium* field. We highlight here four areas of novelty.

- **Crowd-sourced games**. Crowd-sourcing is a strategy to address computationally expensive and difficult problems.[90,91] It allows pieces of computationally challenging tasks to be distributed to large numbers of individuals, each of whom completes a task and returns it to a central system where all solutions provided by the various pieces are compiled. In 2012, Mavandadi et al.[92] and Luengo-Oroz et al.[93] implemented ML coupled with platforms for digital gaming to diagnose *Plasmodium falciparum*, from data in Giemsa-stained blood smears. They both showed that non-medical experts could quickly learn to identify *Plasmodium*-specific patterns, highlighting the value of this approach for telemedicine. Overall, crowd-sourcing is a valuable initiative that facilitates image annotation, acquisition and even model training in ML and DL.
- **'Automated microscopists' and enhanced computer vision**. Prototypes of an automated microscopist for malaria diagnosis started to be introduced as early as 2012.[94–96] In 2017, Eshel et al.[74] evaluated the performance of a commercially available platform, Parasight, in terms of malaria diagnosis, species identification between *P. falciparum* and *P. vivax*, and parasite quantification. Parasight uses a ML algorithm for feature extraction by a support vector machine (SVM) classifier. It was tested in a multisite trial in India and Kenya, for comparison of diagnostic performance with microscopy, rapid diagnostic tests, and PCR. More recently, in 2023, Maturana et al.[53] introduced *iMAGING*, a low-cost robotised microscope coupled with AI-powered image analysis based on YOLOv5x. The whole system was integrated into the *iMAGING* smartphone application.
- **Legal blindness accessibility**. Kumar et al.[97] designed an AI system that can automatically detect malaria parasites in blood samples using hybrid DL. This allows integration of both, detection and classification of *Plasmodium* parasites, and accessible output for visually impaired personnel. This, to our knowledge, is the only work in parasitology, incorporating AI for the purpose of accessibility in terms of disability.
- **Privacy preservation**. We and others have identified that a lack of datasets to train ML and DL models is a barrier to implementation of these technologies. However, when it comes to clinical data, data sharing by medical institutions is regulated by different data protection regulations. Kareem et al.[98] explored federated learning frameworks to overcome this challenge. Federated learning has emerged as a promising approach to enable large-scale analysis across multiple institutions without sharing data.

*Open questions.* AI-driven microscopy has been paradigm-shifting in the field of malaria. It is a field that has paved the way for others when it comes to technology development and testing. There are immense possibilities of how the continuous integration of AI approaches could further advance this field. Below we discuss three.

- Malaria was one of the first parasitology fields to introduce drones to help integrate knowledge about animal reservoirs, vegetation, social dynamics, mosquito populations, and transmission to humans.[99] Integrating microscopy-based knowledge, with this information could better help model and predict transmission patterns and the emergence of antimalarial drug resistance.
- Better predict drug combinations to address the development of drug resistance, as well as better monitoring of features predictive of the emergence of resistance.
- Combining the vast amount of imaging-based and interdisciplinary data from basic and translational science, as well as the clinical field, will make us better able to investigate vaccine design to ensure long-lasting protection against *Plasmodium*. Furthermore, this integration has the potential to guide the design of vaccines that ensure broader protection against multiple *Plasmodium* species.

### Toxoplasma gondii *and toxoplasmosis*

*T. gondii* is commonly transmitted via the oral route through ingestion of undercooked meat of infected animals, exposure to cat feces or contaminated soil (Figure 5B, left panel). It can also be transmitted congenitally and

through organ transplantation or blood transfusion. *T. gondii* displays tropism to neural and muscle tissues. Favoured sites for tropism are skeletal muscles, the myocardium, the brain, and the eyes.[100]

While the overall use of AI is significantly lower than that reported for *Plasmodium*, the range of applications is much more varied. These (summarised in Figure 5B and Table 4) include intra- and extra-cellular tachyzoite identification and viability assessment, analysis of intracellular host–cell protein recruitment by tachyzoites, leukocyte and parasite mapping during brain invasion, and analysis of subcellular features (e.g. mitochondria morphology) of the dormant bradyzoite.

*Intra- and extracellular tachyzoite identification and viability assessment.* Although AI is not used in the clinical diagnostic setting, basic research studies have benefitted greatly from AI implementation. One of the most impactful areas is the use of AI to quantify the number of tachyzoites in host cells. This is of critical relevance due to the following reasons:

- It enables **monitoring of disease progression** in vivo in animal models, in vitro in cultured cells, and in clinical histology.
- It helps identify parasite virulence by enabling the **calculation of multiplication rates**.
- It is key for **drug screening** to monitor effects on parasite load reduction or elimination.
- It enables us to **understand intracellular host cell immune responses** leading to parasite elimination.
- It enables us to **understand host–cell mechanisms such as invasion**, **replication and egress**.

Like *Plasmodium*, AI-based microscopy for *T. gondii* identification has greatly benefited from transfer learning. Two studies, by Yakimovich et al.[101] and Li et al.[102] implemented two different transfer learning techniques for intracellular *T. gondii* tachyzoite identification and viability assessment.

Yakimovich et al.[101] introduced the novel concept of mimicry embedding, which allows transforming datasets so that they mimic verified datasets. The work determined the utility of this approach for analysis of tachyzoite viability in 2D and 3D datasets of multichannel images. They used host cells infected with EGFP-expressing *T. gondii*, labelled for DNA and host cell ubiquitin as a marker of viability. Testing and validation included addition of interferon gamma to induce parasite killing as well as in vivo testing in infected zebrafish. Outcomes were classifications into 4 groups depending on the number of intracellular parasites identified: single viable, single unviable, multiple viable and multiple unviable. Architectures including CapsNet and DropConnect were tested, and the authors concluded that mimicry embedding is useful for higher-expressive-capacity architectures when training on small and unbalanced datasets, such as those common in biomedical sciences.

Li et al.[102] designed a microscopic-macroscopic associated object pulling (M2AOP) strategy and employed a fuzzy cycle generative adversarial network (FCGAN) with transfer learning for *T. gondii* tachyzoite recognition. They used knowledge about the banana- or crescent-shape of *T. gondii* tachyzoites, and that aggregated parasites resemble images of a bunch of bananas, which significantly differ from host cells. The performance of the FCGAN with banana source data was compared to various supervised and unsupervised models, and outperformed unsupervised techniques in both stained and unstained *T. gondii* samples. The FCGAN was tested using images acquired with different microscopes in different countries, and achieved relatively similar accuracy (90–94%). The authors suggest integrating other domain knowledge such as shape, size, colour and statistical properties to identify the most important features defining the success of the FCGAN, so they can be integrated into future DL frameworks to improve prediction.

Besides these studies, in the *Babesia* section below we highlight other work that includes *T. gondii* in DL-aided multiparasite recognition, some of which also implement the use of FCGANs and transfer learning.

*Intracellular host cell-protein recruitment by T. gondii.* *T. gondii* hijacks host proteins from the host cells it invades, for its own survival and growth. Fisch et al. developed the Host Response to Microbe Analysis (HRMAn) pipeline,[103–105] (Figure 5B, middle panel) which uses an ensemble of deep CNNs to extract different features from the input image. This workflow was inspired by AlexNet which utilises a similar approach. HRMAn can learn phenotypes from the data without relying on user input. Moreover, HRMAn is based on the KNIME Analytics platform,[106,107] which facilitates broad usability and easy adaptation to work with other pathogens, as well as software support. The first published version of HRMAn allowed users to choose from a range of analysis methods, from basic quantification of parasite numbers to assessment of spatial distribution of host and parasite proteins. This first iteration was tested within a wide range of experimental setups applied to *T. gondii* and *Salmonella*, measuring parameters such as parasite growth, pathogen killing, and activation of host cell defences.

In 2021 the authors published a new release, HRMAn 2.0,[103] which used a KERAS ResNet50 architecture based neural network instead of the original HRMAlexNet. New features include streamlined user interface that improve

**TABLE 4** Uses of AI/ML/DL-based microscopy and image analysis in the context of parasitology.

| Parasite | Applications of AI/ML/DL | Total studies |
|---|---|---|
| *Plasmodium* spp. | *Diagnostics in RBCs*<br>• Parasite identification and estimation in RBCs for diagnosis, inc. unstained, Giemsa-stained, holographic, infrared spectroscopy, fluorescence, imaging-flow cytometry in clinical samples (165). Some include quantification of RBCs & WBCs.<br>• Identification and classification into parasite stages (rings, trophozoites, schizonts, gametocytes) (55)<br>• Gametocyte identification for transmission-blocking screening (1)<br>• Aggregation and deformability of infected erythrocytes (1)<br>• Assessment of drug-screening efficacy (14)<br>• Speciation between one or more among *P. falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi* (11)<br>*Plasmodium liver stages (host–cell interactions)*<br>• Predicting liver stage development (1)<br>*Pathology*<br>• Vascular players involved in cerebral malaria (1)<br>• Determinants of brain swelling in cerebral malaria (1)<br>*Vector biology*<br>• Identification of infected *Anopheles* mosquitoes (1)<br>*Subcellular structures*<br>• Parasite ultrastructure (2)<br>• Identification of vesicle subpopulations (1)<br>*Novel applications*<br>• Crowdsourcing (2), automated microscopes (2), enabling technologies (1), privacy (1) | 260 |
| *Toxoplasma gondii* | *Host–cell interactions*<br>• Intracellular host protein recruitment to the parasite (3)<br>• Monocytes infiltrating the brain (1)<br>*Subcellular structures*<br>• Mitochondrial morphology classification (1)<br>• *T. gondii* identification based on shape (2) | 7 |
| *Babesia* spp. | *Diagnostics in RBCs*<br>• Parasite identification and estimation in RBCs for diagnosis (based on residual vein or smear samples) (1) | 1 |
| *Cryptosporidium* spp. and *Giemsa* spp. | *Identification in water*<br>• *C. parvum* oocyst identification (2)<br>• *Giardia* spp. cyst identification (2)<br>• Identification and differentiation of both (6)<br>*Identification in feces*<br>• Classification of *Giardia, Cryptosporidium* or both. Differentiation from other helminth eggs or larvae (7) | 17 |
| *Trypanosoma cruzi* | *Diagnostics*<br>• Parasite identification based on morphology inc. unstained, Wright smears, Giemsa smears, etc. (14)<br>• Parasite identification based on motion (3)<br>• Identification of amastigote forms (4)<br>*Pathology*<br>• Identification of parasites in tissues (2)<br>*Drug screening*<br>• Drug activity against parasites with assessment of mammalian cell cytotoxicity (3) | 26 |
| *Trypanosoma brucei* | *Diagnostics in blood*<br>• Parasite identification based on morphology (4)<br>• Parasite species identification (*T. cruzi, T. brucei*, and *T. evansi*) (2) | 6 |

(Continues)

**TABLE 4** (Continued)

| Parasite | Applications of AI/ML/DL | Total studies |
|---|---|---|
| *Leishmania* | *Diagnostics*<br>• Calculation of parasite indexes in macrophages (10)<br>• Identification of promastigote stages (1)<br>• Classification of promastigotes and amastigotes (3)<br>• Classification between *Leishmania* species (1)<br>*Drug screening*<br>• Drug activity against parasites in macrophages (3)<br>*Pathology*<br>• Identification of parasites in tissues (1)<br>• Classification of skin lesions (1)<br>*Advanced methods*<br>• Kinase screening (1) | 23 |
| *Multiparasite* | *Diagnosis of any combination of the parasites above* | 9 |

usability, quality control on images preceding analysis, as well as optimized execution processes to shorten analysis times and improve stability. In terms of biological applications, key advancements included:

- **Improved object detection** through the incorporation of an adapted version of StarDist[108] for nuclei segmentation and a full version of CellPose for label-free cell segmentation.[109,110]
- **Extended analysis into the 3D space**, enabling measurement of vacuole volumes, improving sensitivity of pathogen replication and growth quantification. This also increased the specificity of protein recruitment assessment.
- **Validation with additional organisms** including *T. gondii*, the bacterial pathogen *Chlamydia trachomatis*, and the fungal pathogen *Cryptococcus neoformans*.

In addition to the specific findings in organisms already investigated using HRMAn and HRMAn 2.0, this tool could be of great use for investigation of parasites including *Plasmodium* liver stages,[111–114] *Leishmania*,[115] and *Trypanosoma cruzi*,[116,117] which display complex intracellular interactions with their hosts. Finally, the authors harnessed the power of citizen science, through the establishment of the *Microbe Watch*[118] project on the Zooniverse[119] platform. In this project, specialists and non-specialists can annotate data for training of CNNs.

*Host and parasite players in T. gondii brain invasion.* *T. gondii* disseminates to many organs, including the brain, where it establishes a chronic infection that can persist throughout the lifetime of the host. Active immune surveillance is of vital importance for the host to keep control of *T. gondii*, and this is achieved through many immune players including T cells and monocytes.[120–123] Schneider et al.[124] used intravital microscopy, and light-sheet imaging in transparentised mouse brains at different times of *T. gondii* infection to better understand regional localisation of parasites and immune cells, and monocyte trafficking. Brains were computationally aligned with the Allen Institute annotated brain reference atlas,[125,126] giving insights into the brain-wide pattern of monocyte infiltration. Using ML pipelines for monocyte identification and segmentation, the authors found a correlation between parasite clusters and monocyte presence in defined regions of the brain. Comparing *T. gondii*-infected brains and LPS-treated brains, the authors identified 6 regions in which *T. gondii* induced preferential monocyte recruitment. Among them, infiltration was found to be highest to the olfactory tubercle, which plays a key role in odour-guided behaviour. An additional finding was that monocytes were detected in regions at greater distances from the parasites, raising multiple hypotheses regarding this spatial distribution. Schneider's work is an important proof of concept of the value of ML in the study of parasite tropism in vivo, and its use of powerful tools such as the Allen brain atlas marks an important precedent which could benefit many areas of parasitology.

*Subcellular features: mitochondrial morphology classification in T. gondii bradyzoites.* *T. gondii* contains a single mitochondrion[127] which displays significant plasticity in the actively growing tachyzoite. The *T. gondii* mitochondria morphology is important due to its relevance to various mechanisms including parasite movement between intra- and extracellular environments and its identification as a key drug target.[128–130] While bradyzoites within tissue cysts are considered dormant stages, they are metabolically active.[131,132] Prior to the work of Place et al.,[133] the task of classifying bradyzoite mitochondrial morphologies had not been undertaken. Place et al.[133] used features related to

intensity, size, shape/texture and spatial relation to nuclei to extract mitochondria 'objects' and applied a supervised multinomial logistic regression model to group mitochondria into various morphological classes including blobs, tadpoles, lasso/donuts, arcs and 'other' (Figure 5B, right panel). Most of the identified mitochondria were classified as blobs, while tadpole and arc morphologies—often associated with high metabolic activity- had the lowest incidences. Not only was classification accuracy high, but the time required for mitochondrial segmentation and classification was heavily optimised compared to previous methods.

*Open questions.* Altogether, each of the AI advancements described above in the field of *T. gondii* hold important promise for this and other parasites. Its uses could expand to address open questions in *T. gondii* biology such as:

- **Host range**: integration of AI-guided imaging in multiple hosts could reveal specific features that enable *T. gondii* to infect such a wide range of hosts.
- **Strain-specific virulence**: elucidating cellular, subcellular and immunological factors affecting virulence and clinical presentation.
- **Crosstalk between host and parasite cells**: Understanding immune cell-parasite interactions, particularly in the context of chronic infections.
- **Molecular basis of disease tolerance**: immune cell tracking and spatial characterisation to elucidate disease control (or loss thereof) in immunocompetent and immunocompromised hosts.
- **Intracellular host protein recruitment** across cells of different tissues beyond those already characterised using HRMAn 2.0.

Despite only few studies using AI-powered microscopy for *T. gondii*, this field has implemented some of the most versatile uses. Its complex pathology and multihost invasive capacity make a fascinating subject of research, to which many imaging techniques have been applied. Moreover, this field was pioneer in implementing concepts such as citizen science (through Zooniverse) or make use of state-of-the-art tools such as the Allen brain atlas.

## Babesia *and babesiosis*

*Overview for* Babesia *life cycle.* Like *Plasmodium, Babesia* spp. are vector-borne, and their life cycle involves two types of hosts: mammals and ticks of the Ixodidae family. During a blood meal, *Babesia*-infected ticks inject sporozoites into the host skin, which find their way to RBCs. Transmission happens when the mammalian host is bitten by an infected tick[134] but humans are considered end-points (Figure 5C, left panel). Infection of humans occurs upon entry to tick-infested areas or contact with tick-infested animals. Although over 100 species of *Babesia* have been identified, only a few infect humans.[135]

The spectrum of clinical manifestations is broad, ranging from asymptomatic to severe –and is oftentimes parasite-species related. Therefore, species identification is critical for adequate treatment. In addition, in clinical settings, once the *Babesia* species is identified, parasitemia is used to guide patient treatment. When parasitemia is low (generally under 4%), the disease is managed with antimicrobials, but higher parasitemia (above 10%) is often an indication for RBC exchange[136]—highlighting the importance of accurate identification.

*Diagnosis in RBCs: identification, staging, speciation and classification.* Light microscopy is crucial for babesiosis diagnosis. Nevertheless, important challenges for diagnosis include the relatively low parasitemia levels, and the relative similarities of *Babesia* with *Plasmodium*. One of the forms for definitive *Babesia* diagnosis in microscopy is the 'Maltese Cross' or tetrad form (see schematic in Figure 5C, middle panel), which arises from further division from a 'figure eight' form. The use of AI-powered microscopy is recent in the context of *Babesia* (see Figure 3A), with only six publications reported to date. Only one is specific to *Babesia*[137] while the other five are part of multiparasite classification studies.[138–142] The study by Durant et al.[137] uses DenseNet121 with pre-trained weights from ImageNet for the identification and quantification of *Babesia*-infected erythrocytes. While achieving relatively high classification accuracy, this study reported a high frequency of indeterminate classifications due to confounders like erythrocyte abnormalities, roleaux formations, overlying platelets, debris and precipitates. Additional hindrances were the low number of examples of specific classes resulting in overfitting, and the relatively low availability of images, which do not sufficiently represent the heterogeneity present in clinical settings. The lack of data availability is a problem shared by other parasitology fields, and are highlighted in the respective sections.

*Multiparasite studies: performance for Babesia identification and classification.* Five studies included *Babesia* in their multiparasite DL-based approaches to identify and classify various parasitic organisms of clinical relevance. The first study used deep cycle transfer learning (DCTL) for supervised parasite recognition,[141] and deep transfer graph convolutional networks (DTG-CNNs) based on unsupervised learning.[140] Li et al.'s[141] study aimed to differentiate between three Apicomplexan parasites (*Toxoplasma, Plasmodium* and *Babesia*), applying the knowledge of human experts regarding parasites shape. Namely, that *Toxoplasma* is banana-shaped, *Plasmodium*

is ring-shaped, *Babesia* is pear-shaped, and RBCs are apple-shaped. The DCTL method for supervised parasite recognition is based on the cycle generative adversarial network (Cycle GAN), and consists of 3 subnets: two translators for images in micro (parasites) and macro (fruit shape equivalent) domains, two discriminators and two feature extractors. Key findings specific to *Babesia* were a) a minimum of 10,000 microscopic images were necessary to generate a model with high accuracy and predictability, and b) *Plasmodium*'s and *Babesia*'s similar morphologies (Figure 5C, right panel) are significant challenges for both, human and deep-learning-based classification, with the latter achieving higher accuracy. Li et al.'s[140] study implemented a graph convolutional network (GCN) to extract graph feature representations for multistage *Plasmodium* recognition and used *Babesia* as proof of concept to show differential classification from other parasites.

Tofighi et al.[143] developed a geometric-aware DL method, which Jiang et al.[142] further refined to develop GFS-ExtremeNet (geometric-feature spectrum ExtremeNet) to identify a wide range of variations in shape and size displayed by *Toxoplasma, Trypanosoma* and *Babesia*. GFS-ExtremeNet developed cell priors with convolutional neural networks by learning cell shapes, and U-Nets using DL for cell detection and shape measurements.[144] While the Jiang et al. study demonstrates high accuracy in classification, the authors recognise that to ensure such high accuracy, significant a priori expertise is required for labelling of extreme points that best reflect the geometric features of the target of interest.

The latest work in this field, by Kumar[139] and Anorboev,[138] focused on two methods for overall improvement of identification and classification of 6 parasite types (*T.gondii, Trypanosoma* spp., *Plasmodium* spp., *Leishmania* spp., *Trichomonad*, and *Babesia*), as well as RBCs and leukocytes. Kumar et al.'s work incorporated fine-tuned optimisers to multiple deep learning models. Fine-tuning is the process of adjusting a model to better suit a specific task and dataset, to improve the model's accuracy.[145] This work showed that applying the Adam optimiser to InceptionResNetV2 reached a 99.96% accuracy to classify all organisms, with a similar accuracy to specifically classify *Babesia*. Anorboev's work introduced 'Accentuation Edge via Pixel Value Transformation' as a method to improve parasite classification. This is specifically designed to enhance edges in images by transforming pixel values and focusing on the higher intensity pixels likely representing edges. Incorporation of this method increased accuracy to 99.87%, representing some of the highest classification accuracies in the literature to this date.

*Open questions in diagnosis.* AI is becoming invaluable for *Babesia* diagnostics. If the *Babesia* field were to mimic the strides made in the *Plasmodium* field, three applications for ML and DL will need to be addressed. They are:

- **Model accuracy in *Babesia* staging**: This includes recognition of the multiple forms present in blood, in single infections or co-infections with parasites such as *Plasmodium*.
- ***Babesia* species classification**: *Babesia microti, B. venatorum, B. duncani, B. divergens*, and the currently un-named strain designated M01-type *Babesia* spp. are capable of infecting humans and their severity and lethality can vary greatly. To our knowledge no reports exist for microscopy-based, AI-powered speciation.
- **Incorporation of antimicrobial screening efficiency into models**: Other parasitology areas discussed in this review note how antimicrobial drugs can affect parasite and host–cell parameters (such as morphology, debris, clustering, etc.). These changes can impact the classification and identification accuracies of DL and ML models, and must be incorporated into any model used in a clinical setting.

Finally, although relatively few studies using AI in microscopy are reported for *Babesia*, two factors will likely drive an increase in DL incorporation: (1) translation of easily transferrable expertise arising from the *Plasmodium* field, and (2) the large impact to global human health. This is an increasing concern as there is a high incidence of blood transfusion-transmitted illness, and an increase in the spread of relevant tick populations across the globe. Incorporation of AI-based identification in blood screening could on one hand prevent accidental transmission via this route and could identify asymptomatic carriers for treatment. AI is also an excellent tool for disseminating diagnostic capabilities into areas that may have emergent *Babesia* infections yet lack the microscopy expertise or the human resources necessary for rapid diagnostics.

### 2.2.2 | Apicomplexans and Diplomonads causative of intestinal illness

#### Giardia *(giardiasis)* and Cryptosporidium *(cryptosporidiosis)*

*Overview of life cycles.* *Cryptosporidium* spp. and *Giardia* spp. are intestinal parasites that infect humans and can cause life-threatening gastroenteritis. *C. parvum* is one of the leading causes of severe diarrhoea worldwide, and a significant contributor to mortality in children and immunocompromised patients.[146] Sporulated, thick-walled oocysts are excreted by infected hosts through feces and then contaminate water sources. Parasites are

transmitted through contaminated water (drinking or recreational) and food[146] (Figure 6A, left panel).

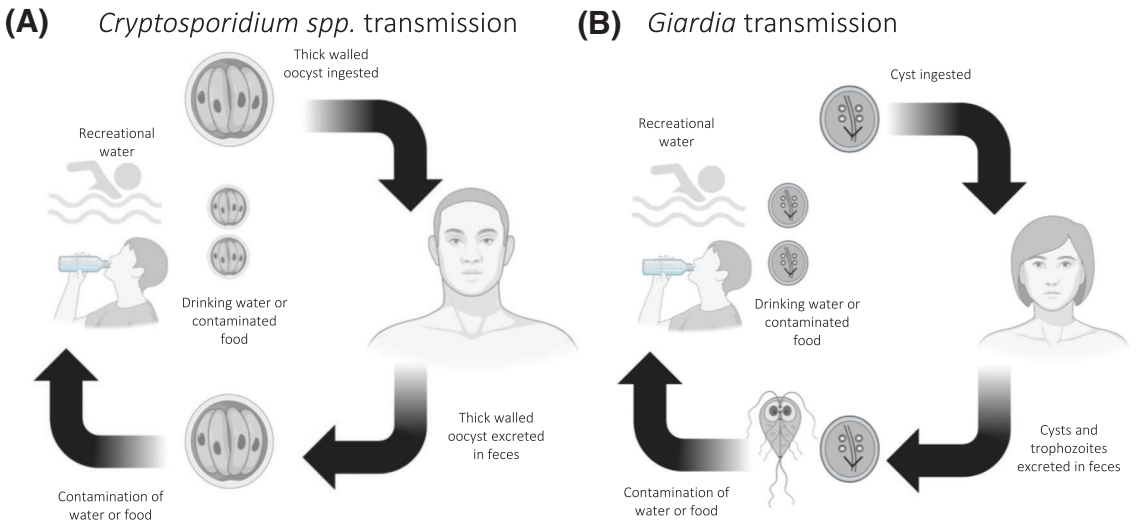
Giardiasis is caused by the diplomonad parasite *Giardia duodenalis*. Like *Cryptosporidium* spp., *Giardia* is transmitted by the fecal-oral route.[147] Its low infection dose and chlorine tolerance allow it to easily infect people through drinking and recreational activities in contaminated water. Cysts (encased forms that can survive outside the body for long periods of time) and trophozoites (the feeding phase of the parasite that predominantly lives in the small intestine) are found in feces and can be used for diagnosis. After ingestion, excystation occurs in the small intestine, releasing trophozoites. These forms multiply by longitudinal binary fission and remain in the lumen of the proximal small bowel. As parasites move to the colon, encystation occurs and cysts are released in feces, where they can readily infect other hosts (Figure 6A, right panel).

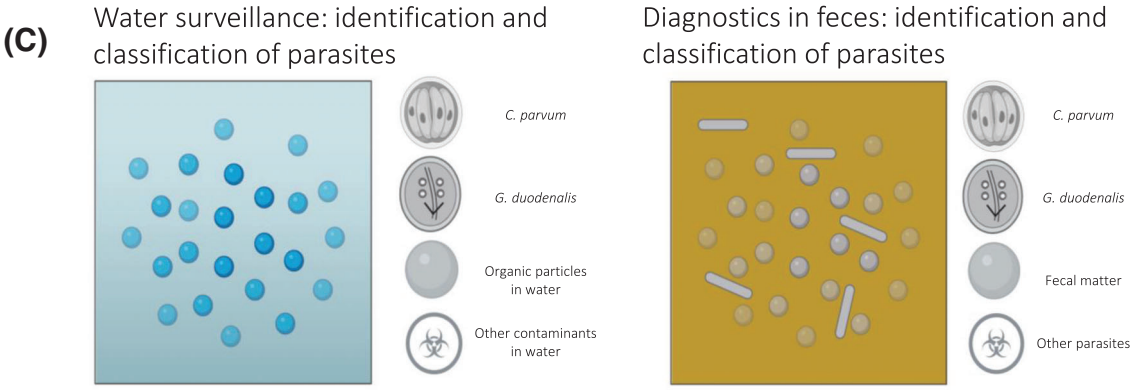
Both parasites are often found in contaminated water sources. Therefore, monitoring water is crucial and has been the focus of public health efforts for decades.[148] Aside from water monitoring, another important area is clinical diagnosis in feces from patients presenting symptoms of gastrointestinal infection. This includes differentiation between parasites (including helminth eggs and larvae), and other fecal debris. This task is extremely challenging and time-consuming, and AI represents a key tool for the future of diagnosis, disease control and disease prevention. We identified 17 studies using AI in the context of microscopy applied to *Cryptosporodium* and *Giardia*. These studies were all either applied for water surveillance[149–159] (Figure 6B, left panel) or for diagnosis, classification and differentiation of various parasites in feces[160–166] (Figure 6B, right panel) (Table 4).

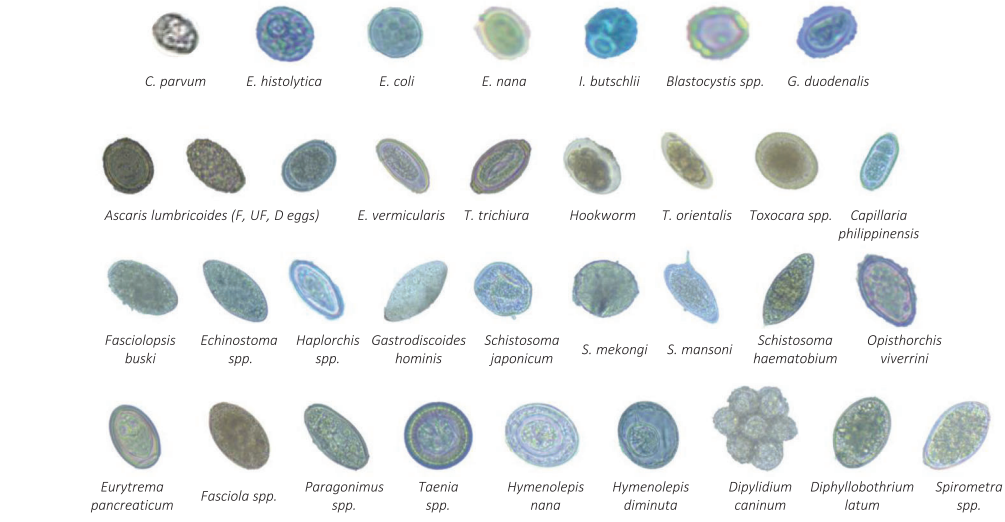
*Water surveillance: identification of* Giardia *and* Cryptosporidium *from other particles.* ML was first reported for the identification of fluorescently labelled *C. parvum* oocysts isolated from water samples.[149] This early work identified key challenges for AI-based identification of parasites in water. AI-powered microscopy has addressed four of those challenges in the six studies highlighted below.

- **High sensitivity required to find *C. parvum* at various levels of concentration in water**: *C. parvum* can be found at various levels of concentration in water. Therefore, a high volume of water needs to be screened to identify contamination. To solve this challenge, several studies[151,154,156] have since incorporated imaging with optofluidic systems to allow for larger water volumes and make water screening more efficient. An example is the work by Luo et al.[157] who introduced MCellNet, a deep learning method which combined with imaging flow cytometry, classifies *Giardia* and *C. parvum* from water. MCellNet uses MobileNetV2 as a building block but outperforms it by cascading six computation-efficient neural network blocks-inverted residual blocks (IRBs) to achieve higher speed, while retaining high classification accuracy.
- **Optimisation of sample preparation**: Water filtration and sample processing steps might destroy or alter parasites, which further complicates diagnosis. Gorocs et al.[155] use holographic imaging that only require mechanical filtration-based pre-concentration techniques, avoiding sample destruction.
- **Identification of parasites from confounders arising from water pollutants, microplastics and other organic non-parasite structures in water with similar shapes**: An example in this direction is the work by Gorocs et al.[155] who developed methodology that couples CNNs and label-free, on-chip holographic imaging for the identification of *Giardia* versus non-*Giardia* objects. The main advantage of this workflow is that the sample preparation is non-destructive, allowing downstream preparation of the same samples for cross-validation with other imaging or complementary research methods. Gorocs' work used DenseNet-121 to perform rapid parasite detection that could be further improved using larger and deeper networks. Challenges identified by this study included the need for faster GPUs to make sample processing more efficient, and the need for improved sensor chips to increase throughput and spatial resolution.[155] Also addressing the challenge of identification and classification, Luo et al.,[156] introduced an improved BiGHAM (Binarized-Greyscale-Hybrid algorithm with Multiregion Binarisation) algorithm. This algorithm identified the radial basis function (RBF) SVM and the bagging decision trees as successful classifiers. Finally, another example is the work by Luo et al.[158] who introduced Siamese-based deep metric learning[167] for parasite identification and classification. This method, achieved the highest outputs for accuracy, precision, and recall so far reported.
- **Portability and accessibility in resource-limited settings**: An example of developments in this area is the work by Koydemir et al.[151] The authors coupled mobile phone-based fluorescence microscopy and ML for the detection and quantification of *Giardia* cysts. The authors custom-designed a smart application (*Giardia* analyser) that wirelessly transmits DNG images to servers for automatic counting. This work used a bootstrap aggregating/bagging approach to classify particles based on 71 extracted features. More recently, Lopez Salguero et al. further optimised DL-based *Cryptosporidium* detection. Their work builds upon various lessons from previous studies and generates a simpli-

# *Cryptosporidium spp.* and *Giardia spp.:* multiple applications



fied tool. It uses Python libraries to label and standardise images to generate input tensors to the YOLOv5 network. YOLOv5s was found to be ideal for systems with reduced resources, requiring the lowest computational cost.[159]

*Clinical diagnosis: identification of* Giardia *and* Cryptosporidium *in feces.* Microscopic identification of parasites in feces is amongst the most challenging parasitic diagnostic tasks, both for human experts, and for AI. This is due to factors including the potentially small parasite sizes, transparency, intermittent shedding in stool, the potential presence of different parasite species (Figure 6D) or life stages, low parasite loads following parasite isolation protocols, and the presence of other fecal components with similar shapes and sizes. Here, we highlight seven tools for image analysis and diagnosis of *Giardia* and *Cryptosporidium* in fecal samples:

- **Invariant-moments adaptive network based fuzzy interference systems (ANFIS)**:[166] optimisation of feature extraction and classification used for pattern recognition. It is based on machine hybrid learning, a technique whereby multiple simple algorithms work together to augment each other to solve problems that alone, neither can solve. This model was ultimately able to achieve 93.5% correct classification of 16 different types of parasites in feces, including *Giardia*.
- **Multiclass support vector machines (SVMs)**:[165] They create decision functions that can accurately predict the class membership of different objects. The work by Avci and Varol (2009) identified the similarity in shapes of parasite eggs as a main challenge that remained to be addressed.
- **Ellipse matching and image foresting transform (IFT)**:[164] When incorporating IFT for image segmentation, and optimum-path forest (OPF) classifier for object recognition, Suzuki et al. solved the challenges of SVMs in parasite egg identification. With higher classification accuracy than any of its predecessors, this tool determined that the best operator tree for multi-parasite classification included 7 descriptors: quantised histogram variants, curvature variance, Fourier coefficients, border/interior pixel classification, tensor scale, Gabor wavelets, and multiscale fractals.
- **Image pixel features to train probabilistic neural networks**:[163] Feature vectors are extracted directly from the image pixel. Saha et al. further improved classification accuracy.
- **Neuro-fuzzy systems**:[162] Nkamgang et al. introduced DL for the first time to this area of parasitology, to address the issue of object classification and identification in complex environments of feces. A neuro-fuzzy system enabled automated detection and classification of 20 intestinal parasites at various developmental stages Figure 6D. This is by far the most advanced method to date. A neuro-fuzzy system combines the learning capabilities of neural networks, and the control capabilities of a fuzzy logic system (built from expert input, data or both). A canny edge detector was used to address the issue of identifying parasites from heterogeneous particles in feces. It was combined with histogram-oriented region (HOG) and linear discriminant analysis (LDA) for feature extraction, and subsequently trained the neuro-fuzzy classifier using the extracted features. Ultimately, 20 rules were generated to explain the classification process.
- **YOLOv4-Tiny**:[161] Moving towards portability and accessibility, Naing et al. trained different YOLO models to recognise 34 different intestinal parasites in simple direct smears and Kato-Katz modified methods. YOLOv4-Tiny was found to produce faster results and use less memory than other models, while achieving over 96% precision (33 of these are shown in Figure 6C).

**FIGURE 6** Overview of *Cryptosporidium* spp. and *Giardia* spp. life cycles and main applications of AI-powered microscopy. (A) *Cryptosporidium* spp. overview of the life cycle. Transmission to humans occurs mainly through ingestion of fecally contaminated water or food. Contamination occurs from sporulated oocysts which are excreted from other infected hosts. Once ingested, excystation occurs, and sporozoites can invade epithelial cells of the digestive tract, where they undergo asexual and then sexual multiplication. Zygotes give rise to thick- and thin-walled oocysts. The former are extracted to the environment through feces while the latter feed an internal auto-infective cycle. (B) *Giardia* spp. overview of the life cycle. Transmission to humans occurs mainly through ingestion of fecally contaminated water or food. Contamination occurs from cysts and trophozoites which are excreted from other infected hosts. Once ingested, excystation releases trophozoites that multiply by longitudinal binary fission in the lumen of the small bowel. Encystation occurs upon travel to the colon. Cysts and trophozoites can be released in feces and are responsible for transmission. (C) AI-powered microscopy has focused on 2 key areas: left panel: identification of *Giardia* and *Cryptosporidium* in water. Key challenges include identification at low concentration levels and classification from other particles found in water including organic particles and other contaminants. Right panel: identification of *Giardia* and *Cryptosporidium* in feces for diagnosis. Key challenges include classification from fecal matter and from (D) other parasitic organisms also responsible for gastrointestinal illnesses. (D) Shows the morphological similarities between various protozoan and helminthic parasites. Naing et al. achieved automatic recognition of these parasites using DL. Images adapted from CDC (*Cryptosporidium* spp.) and from Naing et al. (2022) (reproduced with permission). Figure schematics were generated with BioRender.com.

- **Full workflow automation**:[160] Pointing towards the future of the field, Nagamori et al. evaluated the performance of the Vetscan Imagyst and Ocus40 systems which fully automate the entire workflow for fecal sample evaluation. This includes sample preparation, automated scanning and image acquisition and analysis using DL CNNs. This was a proof of concept for two purposes: comparing the performance of both systems, and testing the potential of pairing up multiple systems to expand throughput and assessment capacities for parasite diagnosis. While this study focused on veterinary samples, the clinical potential of this concept is significant.

*Open questions.* While in our work we highlight only these two parasites when it comes to gastrointestinal illness, huge advances have been done in the field of helminth diagnosis, in particular AI-powered microscopy. We envisage that adoption of important workflows and tailored models already existing in this field will be of great value to *Cryptosporidium* and *Giardia* diagnosis in feces. Equally, *Giardia* and *Cryptosporidium* can be included in AI-powered microscopy-based strategies for water surveillance in other One Health interventions.[168–170] Three areas touched upon by some of the studies we highlighted above, which hold huge potential for the future are: fully automated workflows, integration with mobile technologies, and integrated analysis and processing in more than one location. These three elements open novel possibilities including exponentially expanding diagnostic capacities, reducing sample preparation errors, and fully remote operations in events such as humanitarian crises, where water quality surveillance and disease control are paramount.

### 2.2.3 | Kinetoplastids

Kinetoplastids vary in their form of parasitism, being either free-swimming or obligate intracellular. In their free-swimming forms, they have fast-beating flagella responsible for motility, and an elongated body. This alone, represents an important set of challenges for imaging, image analysis and automation.

#### Trypanosoma cruzi *and Chagas disease*

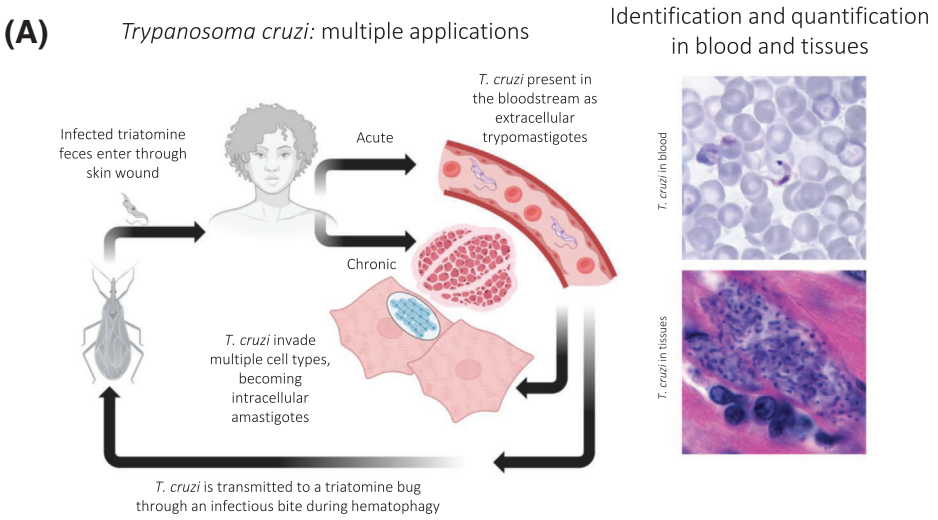
*Overview for* T. cruzi *life cycle.* *T. cruzi*, causative of Chagas disease affects 6–7 million people worldwide, mostly in Latin America.[171,172] *T. cruzi* is transmitted by triatomine bugs, which are haematophagous (Figure 7A, left panel).

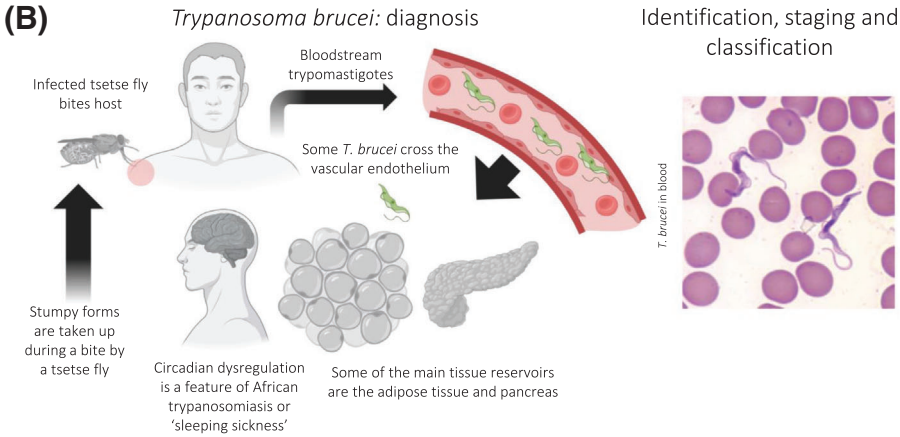
Chagas disease has two stages: an acute stage with different manifestations and parasite circulation in the blood, and a chronic stage, whereby parasites are mostly hidden in cardiac and digestive muscles, often resulting in severe cardiac and digestive pathology. While benznidazole and nifurtimox are highly efficient in parasite clearance if administered during the acute phase of the disease, detection and treatment of chronic disease remains challenging. Therefore, main efforts of AI in the field of *T. cruzi* have been largely devoted to diagnosis in blood and tissues, and pharmacological treatment screening (Figure 3C, Table 4).

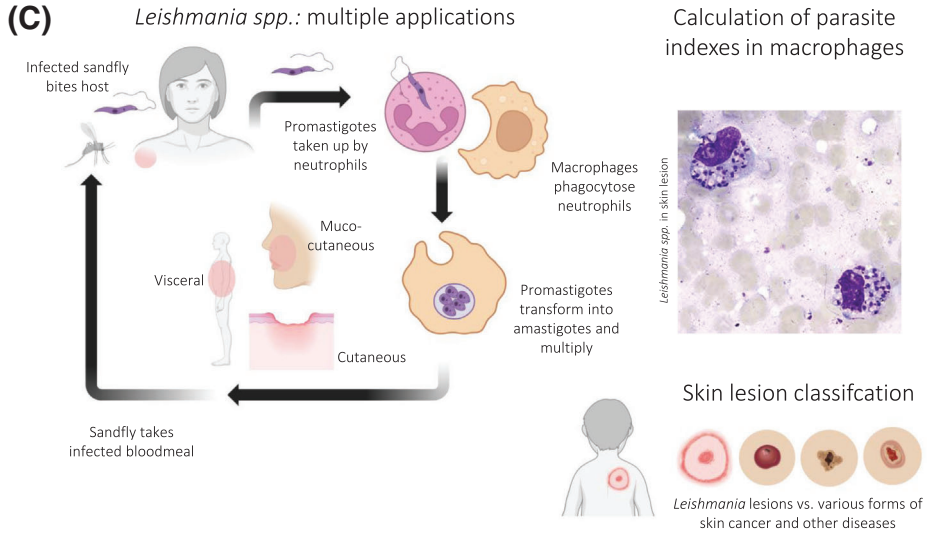
To our knowledge, the earliest work that reported automated approaches for Chagas disease diagnosis dates to 2004,[173] with the purpose of high-throughput screening of trypanocidal drugs. Since then, the field has explored three key categories:

*Diagnosis based on* T. cruzi *morphology in blood.* One of the most common ML/DL application in *T. cruzi* studies has been automated detection of the blood borne trypomastigote stage (present during the acute phase of Chagas disease) from blood smears or fluorescent reporter parasites. Early efforts focused on developing and improving pipelines for parasite segmentation and identification in the presence of other cells in the blood (Figure 7A, right panel, top). Since 2014, several groups have used ML algorithms such as Gaussian-based methods,[174] AdaBoost and SVMs,[175] Chaotic Cuckoo search[176] and random forest algorithms to count trypomastigotes in mobile phone images.[177] Since 2020, key DL developments for the *T. cruzi* identification and segmentation tasks include CNNs such as MobileNetV2,[178] FCNN (a residual CNN for semantic segmentation),[179] and Res2 SVM.[180] The latter have shown major improvement in parasite identification, reporting significantly higher sensitivity and specificity compared to earlier efforts. Notably, many authors recognise the value of methods generated in the *Plasmodium* field, which served as inspiration for many of these models. Nonetheless, due to morphological differences between both parasites, most models had to be specifically designed, tailored or trained for optimal performance in *T. cruzi* diagnosis.

*Diagnosis based on* T. cruzi *motility in blood.* Another large area of AI-powered microscopy for *T. cruzi* diagnosis incorporates motility, either standalone or in combination with morphology analysis. Parasite motility in fresh blood is a key feature for identification and early detection. Romero et al.[181] first reported using digital holographic microscopy to identify phase changes in regions where parasites move. Since then, other tools developed for this purpose include cubic higher-order local autocorrelation (CHLAC) models used not only for parasite identification, but also for trypanocidal drug screening.[182] Other examples are collateral motion saliency-based models that identify collisions with neighbouring cells[183] and Long Short Term Memory (LSTM) convolutional autoencoders that identify abnormal motion patterns.[184] More recently, trajectory-driven



models helped identify collateral, fluctuating, and pan-tilt-zoom motion.[185] Despite these exciting advances, further optimisation is still required to better understand *T. cruzi* motility in the context of diagnosis and drug screening.

*Detection of intracellular* T. cruzi *forms in vitro and in tissues.* The third large group of AI-based research in *T. cruzi* has focused on detection of intracellular amastigote stages, which are most relevant for the chronic stage of Chagas disease. This work has been mostly done in in vitro cultures. The main challenge for detection is parasite identification within cells in dense environments. This is particularly problematic for *T. cruzi* in that parasites undergo binary fission and large-scale replication within the host cell (Figure 7A, right panel, bottom). Early work in this field focused on optimising segmentation tools to evaluate trypanocidal drug effects in terms of *T. cruzi* survival rates, and host cell cytotoxicity.[186–189] More recent work has gone a step further, using convolutional neural networks for *T. cruzi* amastigote detection in histopathological images of infected tissues.[190,191] Altogether, they still report significant challenges and recognise that AI applications for chronic Chagas disease research is an area that merits further work.

Trypanosoma *species classification.* The work of Kittichai et al.[192,193] explored hybrid models for deep metric learning (DML) and vector database-based image retrieval systems to identify and categorise trypanosomes of different species. Unlike object classification where prediction relies on class probabilities, DML measures true positives based on similarities between the query image and a well-trained database. Key achievements of this model were accurate discrimination of *T. cruzi, T. brucei* and *T. evansi* and identification of any of these species when in environments where other parasites such as *Leishmania* spp., *Toxoplasma* spp., or *Trichomonas* spp. were present. Additional advantages include DML's robustness in terms of performance upon variations in the dataset, resilience to class imbalances, and better ability to manage large-scale datasets with a high volume of classes. DMLs also have the capacity to generalise to novel classes that were not encountered during training, which is advantageous in scenarios where new classes may consistently emerge. Altogether, species classification is highly relevant due to the importance of early detection, and choice of adequate treatment.

*Open questions in diagnosis.* Keys aspects of diagnosis that remain to be addressed through ML and DL for Chagas disease include:

- **Strain diversity and virulence**: Exploration of DL-based models for identification and characterisation of *T. cruzi* strains.
- **Immune response mapping in chronic disease**: A combination of AI-powered image analysis and light-sheet imaging would help better characterise immune cell populations in the context of chronic disease establishment. This is similar to work performed in *T. gondii* to characterise spatial distribution of monocytes and parasites,

**FIGURE 7** Overview of *Trypanosoma cruzi, Trypanosoma brucei*, and *Leishmania* spp. life cycles and main applications of AI-powered microscopy. (A) *T. cruzi*. Left panel: Trypomastigotes enter the human host through feces deposited near a bite by the triatomine insect vector. Inside the host, trypomastigotes invade cells near the bite site, and differentiate into intracellular amastigotes which multiply by binary fission. The differentiate into trypomastigotes to the bloodstream and can invade tissues at multiple locations, where they again differentiate into amastigotes. Triatomes can take up circulating trypomastigotes upon a bloodmeal. AI-powered microscopy has been used for multiple applications in *T. cruzi*. Right panel: Two of the main applications include parasite identification in blood smears (Giemsa-stained blood smear, top), and amastigote identification and quantification in tissues (H&E stain tissue biopsy, bottom). Images are courtesy of the CDC. (B) *T. brucei*. Left panel: Infected tsetse flies inject metacyclic trypomastigotes upon an infectious bite during a bloodmeal. Parasites transform into blood trypomastigotes, which divide by binary fission. They are carried to various locations in the body through circulation and are capable of traversing the blood vasculature and establish large tissue reservoirs in organs such as the adipose tissue and pancreas. They can also invade the brain, and cause circadian cycle dysregulation resulting in the common name for the disease, 'sleeping sickness'. In the blood, two main forms exist: slender and stumpy forms. Stumpy forms are responsible for transmission to the tsetse fly. Right panel: The main application of AI-powered microscopy for *T. brucei* has been for identification and species classification from other *Trypanosome* spp. Image shows a Giemsa stained blood-smear showing two *T. brucei* parasites. Images are courtesy of the CDC. (C) *Leishmania* spp. Left panel: *Leishmania* is transmitted by the bite of an infected phlebotomine sandfly which injects promastigotes during a bloodmeal. At the site of infection, promastigotes are ingested by neutrophils which are involved in the recruitment of macrophages, which in turn become infected. In macrophages, parasites (amastigotes) multiply by division. Depending on a combination of host and parasite factors, leishmaniasis can be cutaneous, muco-cutaneous or visceral. Sandflies become infected by ingesting infected cells during a blood meal. AI-powered microscopy has been used for multiple applications in *Leishmania*. Right panel: Two examples of applications are quantification of amastigotes within macrophages to calculate the parasite index (top panel), and the classification of cutaneous lesions to differentiate them from other skin pathology including other wounds and cancer (bottom panel). Images are courtesy of the CDC. Figure schematics were generated with BioRender.com.

- **Immune response variability**: Understanding of host responses to varying clinical presentations and/or disease phenotypes.
- **Organ-specific pathogenesis**: Understanding pathogenesis at the organ, cellular and subcellular levels using tools such as HRMAn 2.0 could be useful to understand the basis of phenotype variability.
- Incorporation of all points above for assessment of **drug screening strategies**.
- Development of models for **lineage tracing and cell cycle progression assessment**.

AI-based microscopy has been heavily incorporated into *T. cruzi* research, and will likely continue to expand to other questions, and other microscopy techniques. Ultimately, as for other pathogens, it is likely to play a major role in drug treatment development and vaccine development.

### *African trypanosomes and African trypanosomiasis*

*Overview of* T. brucei *life cycle.* African trypanosomes (*T. brucei, T. vivax, T congolense, T. evansi, T. equiperdum, T. suis* and *T. simiae*) cause African trypanosomiasis in humans and/or animals. Collectively, they are biologically transmitted by the bite of an infected tsetse fly (*Glossina* spp.)[194] (Figure 7B, left panel). Tsetse flies are haematophagous, and an infectious bite results in the injection of metacyclic trypomastigotes that transform into bloodstream trypomastigotes. In the case of *T. brucei*, these extracellular flagellated parasites can traverse the blood vasculature and establish tissue reservoirs of different density and duration across organs.[195–202] In this context, a signature pathology of Human African trypanosomiasis is severe alteration of the circadian rhythm.[203,204] This led to its more commonly referred name, 'sleeping sickness'. Conversely to *T. brucei*, species like *T. vivax* and *T. congolense* are intravascular and sequester to the blood vasculature.[205–207] In this respect, correct identification, parasitemia estimation, and speciation is of great importance to improve clinical and veterinary outcomes. *T. brucei, T. congolense, T. vivax* and *T. evansi* display different morphologies and motility patterns which might be detectable by AI.

The multiple life cycle transitions of *T. brucei* that occur within the insect vector have been a topic of major interest for decades.[208] While in terms of transmissibility, *T. brucei* is historically classified into two morphologically distinct stages known as stumpy and slender forms, recent work suggests there is an additional layer of parasite heterogeneity in the mammalian host.[209] Several molecular signatures, and morphological and motility patterns have begun to be identified, and AI will likely play a key role in helping us better understand the relevance of potential intermediate forms, and parasite variability based on tissue tropism.[196,197,200–203,209–211]

*Diagnosis in blood: identification, classification and staging.* Five publications have focused on AI for either *T. brucei* (Figure 7B, right panel) or *T. evansi* identification or stage classification (Table 4), or species identification. We found no AI-related publications using microscopy for other African trypanosome species.

The first set of papers addressed the identification of *T. brucei*[212,213] or *T. evansi*[214] in blood smears. Jung et al.'s work[213] addressed the issue of sensitivity limitations of parasite identification at low parasitemia during chronic infections. They built a CNN-based classification model from unstained thick bloodsmears. A ResNet18 model was applied on a pre-processed dataset (involving cropping and thresholding), resulting in the classification of patches of unstained smears into binary classes based on parasite presence or absence. Jomtarak et al.[214] developed a mobile app 'CIRA Bot app' based on YOLO versions to detect slender and stumpy forms of *T. evansi* in thin blood smears. CIRA Bot is a line chatbot that operates a smartphone's camera to capture images and serves as a local server. The performance of various YOLO models was tested, and YOLOv4-tiny neural network model was found to give the highest overall identification accuracy, with higher prediction of slender (97%) than stumpy (90–93%) forms. Class imbalance and the large morphological variation attributed to slender forms were indicated as potential explanations for this difference.

The second set of papers focuses on the generation of a hybrid model, that combines deep metric learning and vector database-based image retrieval for the categorisation of trypanosome species including *T. cruzi, T. brucei*, and *T. evansi*,[192,193] even in the presence of other related and non-related pathogens such as *Leishmania, Trichomonas*, and *Toxoplasma*. The latter developments for categorisation have important implications not only for the detection of co-infections, but perhaps equally relevant, for the study of parasite heterogeneity within the mammalian host.

*Tryp image dataset generation to aid DL training for diagnosis.* Anzaku et al.'s[212] work aimed to address an important barrier to AI and specifically, to DL: the lack of curated datasets. They generated the Tryp dataset, using unstained thick blood smears, which provides bounding box annotations for regions containing *T. brucei brucei* parasites, as well as negative blood samples. To validate whether the image diversity included in this dataset was valuable for DL model training, the authors designed a validation process using three DL models: Faster R-CNN, RetinaNet

and YOLOv7.[212] The three models were evaluated in terms of performance, with varying degrees of accuracy and speed for each model.

*Open questions in diagnosis.* As for other blood-borne parasites, AI has significant potential for African trypanosome diagnosis. Building on the achievements of tools and applications developed for both, Apicomplexans and other *Trypanosome* species, such as *T. evansi* and *T. cruzi*, keys aspects of diagnosis that remain to be addressed through ML and DL for African trypanosomes include:

- **Assessment of model performance for African trypanosome staging into slender and stumpy forms**: This would guide evaluation of transmission potential and discovery of monomorphic strains.
- **Development and assessment of model performance for identification of 'intermediate forms'**: This would bring better understanding of parasite heterogeneity in the context of virulence, tropism, cell cycle progression, and transmission.
- **Development of models incorporating motility as a feature for identification, classification and species identification**: This would improve diagnosis in wet samples.
- **Development of models for lineage tracing and cell cycle progression assessment**: this would increase our knowledge on parasite biology.
- Incorporation of all points above for assessment of **drug screening strategies**.

Notably, DL would allow for characterisation of vast numbers of parasites for each of the points below, which is a current bottleneck in the field.

## Leishmania *spp. and leishmaniasis*

*Overview of* Leishmania *life cycle.* Leishmaniasis is caused by obligate intracellular protozoan parasites of the genus *Leishmania* which are transmitted by over 90 different species of phlebotomine sandflies.[215] Infected sandflies (Figure 7C, left panel) use the proboscis to inject *Leishmania* promastigotes, which are internalised by phagocytic cells at, or close to, the site of injection. While phagocytic cells aim to control the infection through various mechanisms involving neutrophils and macrophages, it is believed that *Leishmania* spp. can disseminate within the host due to a 'Trojan horse' mechanism derived from this very process.[216–218] Inside macrophages, promastigotes transform into tissue stages called amastigotes, which divide asexually and burst out of the cell for auto-infection. Forward transmission occurs when a sandfly ingests macrophages infected with amastigotes.

Based on a yet poorly understood complex combination of parasite, vector and vertebrate host factors, leishmaniasis can develop into tegumentary (cutaneous, mucosal and cutaneous diffuse forms), or visceral[219] classes. Early diagnosis and accurate species identification is essential to facilitate clinical management and guide treatment options, because of the large differences in treatment course and efficacy required for the various forms of the disease.

Key areas of AI-powered microscopy and image analysis have been diagnostics and drug screening, followed by pathology-based classifications, and advanced methods such as kinase screening.

*Diagnostics: calculation of parasite indexes in macrophages.* The first reports of AI applied to *Leishmania* image analysis occurred in 2013. The most common application focuses on identification, segmentation, and quantification of *Leishmania* amastigotes within macrophages (Figure 7C, right panel). Amastigotes are spherical to ovoid in shape, and have a prominent kinetoplast. Early AI-based tools faced important challenges: low detection accuracy, poor performance under illumination non-uniformities/high prevalence of overlapping elements, and unmixing problems.[220–225] Moreover, computational requirements were limiting. Even though these studies were limited, they became the steppingstone for tackling more complex questions relevant to *Leishmania* epidemiology. Two examples are:

- The development of algorithms to investigate the effects of antileishmanial treatment on parasite survival and macrophage toxicity.[226,227]
- The optimisation of detection and classification methods for with primary macrophages instead of cells lines,[226] and macrophages of different tissues or wound lesions.[228–230] This adds significant complexity compared to previous tasks due to the complexity of environments found in tissues.

The state-of-the-art DL methods used today address previous challenges for parasite identification in macrophages in vivo, in situ, and in vitro. Moreover, they have achieved successful classification into intracellular and extracellular forms, and life stage identification.[231–235] Here we discuss three tools:

- **FiCRoN**[232] is based on fully convolutional regression networks and has better performance in

quantification and classification tasks than YOLO, CellPose and CellProfiler.
- **LeishFuNet**[233] can be used in the context of telemedicine, and for the first time uses augmentation techniques, as well as implementing Grad-CAM to demonstrate the model's interpretability.
- **DeepLeish**[234] determined that the YOLOV5 model outperforms other models in terms of MAP scores and precision and recall metrics.

Together, these models are all highly relevant for early detection of the disease, facilitating timely decisions on treatment course. However, challenges remain including the lack of image repositories, lack of availability of large datasets for DL training, and class imbalances, which result in a lack of generalisability of the models. Clear immediate future directions in this field include training models to differentiate between visceral, cutaneous, and mucosal variants, possibly using cascaded networks.

*Host–cell interactions: understanding* Leishmania *survival in macrophages.* Going a step further in understanding the 'black box' of *Leishmania* pathology, two studies used ML to investigate specific indicators controlling *Leishmania* survival within host macrophages or during their uptake. One study used TissueFAXS cytometry and the DotFinder algorithm to assess F4/80 and CD11b expression together with parasite viability markers to explore a potential link between macrophage profiles and parasite survival.[236] A more recent study used an elastic net regression algorithm (kinase regression or KiR) to investigate the underlying signalling mechanisms controlling *Leishmania* uptake by macrophages.[237] The robustness of both tools remains to be tested under multiple clinical conditions, but they highlight the potential of AI-aided imaging to better understand the specific mechanisms governing pathology, disease progression and severity.

*Lesion classification.* A CNN-based model was generated to help distinguish CL lesions from other types of cutaneous lesions such those present in melanoma and Hansen disease.[238] This raises an important topic for AI in the medical field, which is the crosstalk between different medical fields to facilitate knowledge and resource integration. This could be via the incorporation of training models to encompass a wider range of diseases and pathology with similar symptomatology and the need to share imaging databases, which we will further discuss in future sections of this review.

*Harnessing the strength of imaging beyond fluorescence microscopy.* Outside of the clinical setting, studies have gone beyond conventional fluorescence microscopy and applied ML and DL to techniques such as Fourier transform infrared (FTIR) microspectroscopy and Matrix-assisted desorption ionisation-mass spectrometry imaging (MALDI-MSI). These were used for three applications:

- Discriminating between uninfected blood and blood infected with different parasite species in canines (i.e. *T. evansi* or *Leishmania* spp.).[239]
- Classifying between amastigotes and promastigotes and discriminating between infected and uninfected macrophages.[240]
- Discriminating between uninfected, infected untreated and infected treated lesions in mice.[241]

These hybrid techniques are rapid and cost-effective which allows analysis of otherwise unmanageable data amounts arising from spatial and spectral information from each sample. Clear future directions include the possibility of investigating signatures specific to each *Leishmania* spp. as well as biochemical changes throughout infection that might give better insights into the mechanisms governing the wide range of symptoms and disease progression characteristic of this disease.

*Open questions in diagnosis.* Great advances have been made for *Leishmania* amastigote identification in macrophages. Important open questions that could be addressed soon, especially in view of tools that have been developed in this and other parasitology fields are:

- **Molecular mechanisms of disease establishment**: Determining tissue-specific, cellular and subcellular parameters governing symptomatic and asymptomatic infection.
- **Lesion identification and classification**: through expansion beyond cutaneous leishmaniasis, this would be key for the early diagnosis of muco-cutaneous and visceral leishmaniasis.
- **Portability**: Development and deployment of DL-based tools to resource-poor settings where *Leishmania* is endemic.
- **Diagnosis sensitivity**: Parasite identification at low parasitemia for detection of latent, subclinical, and asymptomatic cases.
- **Host cell-parasite interplay**: understanding of the Trojan horse mechanism enabling *Leishmania* dispersion beyond the site of infection.

Further work using AI will be valuable to guide personalised treatment, with the end-goal of reducing morbidity and mortality associated with leishmaniasis.

# 3 | CHALLENGES AND FUTURE DIRECTIONS FOR AI-POWERED MICROSCOPY IN PARASITOLOGY

## 3.1 | Overview of current achievements

AI applications have moved from image analysis for single species parasite identification, to the implementation of advanced algorithms for multiparasite, multistage, or multispecies differentiation. These complex analyses are possible even in highly heterogeneous and complex samples including feces, water, and tissues. We identified four clear paths of growth at the software level. The first is the ready adoption of DL to solve issues originally addressed by ML (see trend in Figure 3). Second is the implementation of DL-based tools that are less computationally and temporally intensive, without compromising on detection power. In this context, exploring novel tools such as NanoPyx[242] will be an interesting future direction. Third is the integration of DL with mobile technology to allow for deployment in field and remote settings. Fourth is the integration of automated workflows (exemplified by the Parasight,[74] iMAGING[53] or the Vetscan Imagyst[160] systems) allowing for simultaneous acquisition in multiple locations, reducing sources of variability at various steps of the imaging workflow. Given the global effort invested in the field of AI-powered microscopy and parasitology (Figure 8A), the possibility of international efforts harmonising through AI will be an exciting avenue to explore.

Beyond these achievements, our analysis of almost 400 papers for this review reveals three main features that make ML and DL an invaluable addition to microscopy-based parasitology:

- They can learn and detect complex relationships between features in data.
- DL models are hugely scalable, able to learn from a huge range of experiences and make accurate predictions.
- DL models can learn in a data-driven way, requiring very little human intervention to train them.

Altogether, for clinical applications, the strongest of which is diagnostics, the field has done an impressive job in developing and keeping up with latest AI technologies. This has included successful implementation of many state-of-the-art methods. These include but are not limited to fine-tune optimisers to DL models, mimicry embedding, Siamese-based DL, machine hybrid learning, multiclass SVMs, neuro-fuzzy systems, deep metric learning, and LSTM convolutional autoencoders. One of the fields that is showing great promise is transfer learning. Its importance was recently reviewed by Feng et al.,[45] highlighting the value of integrating human expertise to DL.

## 3.2 | Overview of current challenges

The use of AI in parasitology faces challenges that are shared with other biomedical fields. Here we highlight two such challenges:

### 3.2.1 | Low data availability

High-throughput technologies represent a major opportunity for image generation, and in this sense, various studies assessed in this review already implement tools such as imaging flow cytometry, and high content screening. We have previously reviewed microscopy in the context of big data and parasitology, as well as potential for adapting low-throughout imaging technologies to yield high-throughput outputs.[2] Altogether, increasing image input per study will improve DL training.

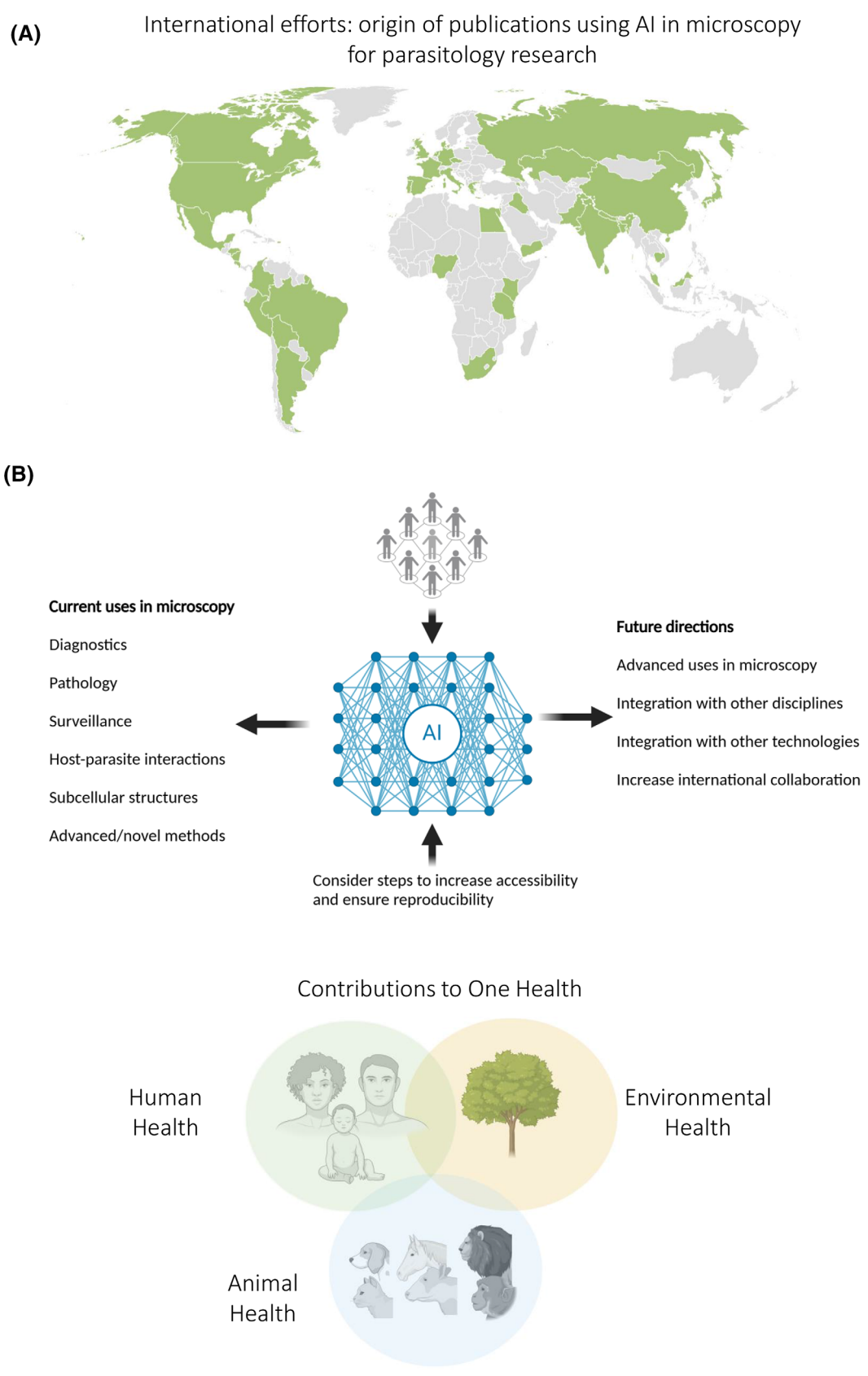
Another way of increasing throughput is through self-driving laboratories. Autonomous and automated imaging hold great promise for increasing data acquisition and processing capacities.[243,244] However, to our knowledge, no collaboration or effort of this level exists yet in parasitology.

### 3.2.2 | Reduced number of public image databases

While high-throughput imaging could increase the sheer number of images acquired per study, those images might not be efficiently accessed because there is a low number of public and centralised image repositories or archives. Given that microscopy, parasitology, and public health have been tightly intertwined for almost a century, there should be no scarcity of images or samples per se. Yet most images are either not published, not openly available, not easy to find, or not sufficiently annotated.

*Examples from other technologies: the value of VEuPathDB for parasitology*

A perfect example of the value of public databases and community effort to accelerate scientific discovery in infectious diseases is VEuPathDB,[245–247] which centralises 'omics' data. This is a tool that for over 20 years has built a Findable, Accessible, Interoperable, Reusable (FAIR) access infrastructure supporting omics datasets and the tools to analyse and navigate them. VEuPathDB has been critical for our understanding of parasitic organisms, and the discovery of critical medication, vaccines design, and drug resistance surveillance.[248] A similar tool for imaging, and/or its incorporation to existing databases such as VEuPathDB would be instrumental.



*Global efforts to establish imaging archives*

The importance of imaging databases to life sciences has been the topic of discussion of various seminal studies.[249–252] Outside parasitology, significant efforts have been made by the microscopy community for the establishment of easily searchable image repositories. Examples include the Image Data Resource[253] and the EMBL's Bioimage Archive.[254,255] The same is true for the development of platforms for image-based model training, such as the BioImage Model Zoo.[256] Therefore, fostering interactions with these developers and other scientific communities making use of these platforms is an important step. Within parasitology, some efforts in this direction have begun, including work highlighted in this review such as the development of the Tryp database,[212] or the Microbe Warch project in Zooniverse.[118,119] Further efforts in this direction will be paramount in the future, especially as DL model implementation increases, and greater amounts of data become pre-requisites.

## 3.3 | Insights from other biomedical fields and applications to answer outstanding parasitology questions

Although most of the AI-based microscopy research has been in the context of light microscopy for diagnostics (Figures 5–7), many other imaging techniques have been incorporated into parasitology research.

These include force spectroscopy, hybrid technologies (e.g. organs-on-chip and microfluidic devices), advanced fluorescence microscopy (e.g. super-resolution microscopy, fluorescence dynamics, time-lapse imaging, expansion microscopy, lightsheet microscopy, and intravital microscopy), medical imaging (e.g. ultrasound, photoacoustic, x-ray, PET, SPECT, CT), electron microscopy (e.g. TEM, SEM, STEM, cryo-EM, and CLEM), and soft-X-ray microscopy. They have allowed us to address complex biological questions in several subfields and across scales. We and others have previously reviewed advances in these fields for in vitro and in vivo imaging.[1,2,4,6,7,18,257–259]

Although AI-driven image acquisition and image analysis for each of these methods has been reported in other fields, many remain to be implemented for in parasitology. Although the possible number of applications is vast, below we specifically discuss four cases relevant across parasitology fields.

### 3.3.1 | Understanding parasite ultrastructure: AI-powered EM

Electron microscopy (EM) is unrivalled in its ability to provide structural information. Its use in parasitology has been extensive, and we refer readers to several key reviews covering this topic.[5–7,257] Together with conventional TEM and SEM, volumetric EM tools have led to a better understanding of parasite morphology, life cycle, host cell-parasite interactions, and structural biology (reviewed by Cooper et al.[7]). Even in 2D, analysis of EM-derived images is complex due to subtle details that make segmentation difficult (especially for novel or complex features) and for the faint difference in contrast in EM micrographs. This is exponentialised in 3D image analysis where gigabytes or even terabytes of data can be easily generated, making manual segmentation highly complex or almost impossible.

DL has addressed several challenges related to segmentation and classification in EM images using CNNs, RNNs, transformers, transfer learning and deep reinforcement learning (reviewed by Chen et al.[260]). While many valuable tools have been generated, we highlight four recent developments:

- **FAMOUS**: Nesic et al.[261] introduced fast automatic outline segmentation (FAMOUS), which combines organelle detection, image morphology detection and 3D meshing. They tested this on HeLa cell datasets acquired with FIB-SEM, and on yeast cells acquired with transmission ET. FAMOUS demonstrated that it can handle large datasets (in the terabyte range) in relatively little time, greatly increasing throughput while achieving high classification accuracy.
- **Cryo-CARE**: Buchholz et al.[262] introduced content-aware image restoration for electron microscopy. In cryo-TEM, segmentation is challenging because of the low signal-to-noise ratio caused by the limited electron

**FIGURE 8** Overview of current and future directions of AI-driven microscopy and image analysis in parasitology. (A) Our current implementation of AI in microscopy for parasitology research is the product of international efforts involving at least 45 countries (based on the location of first and last authors of the 350 publications included in this work). (B) To this day, most AI-driven microscopy advances in parasitology are the result of international collaboration and have been made in the field of diagnostics, followed by water surveillance, pathology, host–parasite interactions, subcellular structures and novel applications. Future directions include incorporation of AI for analysis of advanced imaging methods, as well as better integration with other disciplines and other technologies. Altogether, AI-driven microscopy has significant potential for addressing current bottlenecks in One Health outcomes. Figure schematics were generated with Datawrapper.de and BioRender.com.

dose. Cryo-CARE builds on CARE[263] and allows training content aware restoration networks for cryo-TEM data, both for 2D and 3D.

- **MorphoFeatures**: Zinchenko et al.[264] introduced a tool for fully unsupervised characterisation of cellular shapes and ultrastructures of a whole body of a nereid worm. Data was mapped to a cellular atlas of this organism,[265] allowing for a co-analysis of whole -body gene expression maps.
- **Empanada and MitoNet**:[266] Empanada and MitoNet are deep learning tools for segmentation of 3D data sets for subcellular structures based on a vast set of EM datasets.

While this list is not exhaustive, these tools show a range of possibilities for parasitology. These range from understanding complex host–parasite interactions in full organs or organisms, to the easier investigation of parasites and their host cells in their native state.

### 3.3.2 | Understanding host–parasite interactions: pathology, parasite tropism and the establishment of parasite reservoirs

The study of host–parasite interactions has been the subject of great interest, and has been based in the exploration of animal models, insect vectors, organoids, hybrid platforms, and in vitro models. These have been paramount to our understanding of parasite-mediated pathology, tropism, immune evasion mechanisms, and long-term sequelae of infection. The number of imaging methods that have been used in this context is extremely diverse leveraging every advance in microscopy. Aside from slide screening of tissue sections, studies in model organisms use intravital microscopy, lightsheet imaging of transparent samples, fluorescence lifetime in imaging, and photoacoustic imaging, among many others. A wide range of ML and DL methods have been implemented for each of these techniques in other scientific areas. Readers are referred to the recent work by Volpe et al.[267] for a comprehensive review of advances in DL for multiple biomedical applications. Below, we highlight six examples which we envisage would be of interest for parasitology.

- **Virtual staining**[268–270] of tissue sections to gain insights into infection-related pathology across organs from existing samples. Histology and histochemical analysis of tissue sections is a gold standard for disease diagnosis. Virtual staining can be done in unlabelled tissue sections and involves the use of DNNs to transform images of label-free tissues into various stains. DL can also be used on stained tissue sections to transform one type of stain to another.
- **Cell Painting CNN**[271] for phenotypic characterisation of cells and tissues in control, infection and drug treatment conditions. Cell Painting[272] is a morphological profiling assay that multiplexes multiple channels and dyes, resulting in eight relevant cellular components or organelles, which outperforms conventional analyses.
- **DL and label-free autofluorescence lifetime imaging**:[273] Fluorescence lifetime in imaging (FLIM) can provide valuable information about the metabolic state, pathological state, or constitution of cells and tissues, based on endogenous signals. FLIM has been valuable for a wide range of biological questions relevant to parasitology, for instance, macrophage polarisation.[274] One of the challenges of FLIM analysis is the limitation of acquiring cellular level information. The DL-aided generation of FLIM-based virtual H&E stains has been a way to overcome this issue.
- **3DVascNet**:[275] The blood vasculature is key for the distribution of many parasites from one anatomical location to another. For some parasites that sequester or cytoadhere to them, these locations are of great clinical relevance. Manual quantitation of vascular parameters is extremely laborious. For techniques such as lightsheet which yields large volumes of data, it is prohibitive. 3DVascNet is a DL-based software for automated segmentation and quantification of 3D vascular networks.
- **Deep3P**:[276] Intravital microscopy has enabled us to elucidate vital events related to parasite-mediated invasion and colonisation of the host. These include *Plasmodium* merosome egress from the liver,[277] *T. brucei* tropism,[196,198,200,202,278] or *Leishmania* interactions with neutrophils and macrophages (the 'Trojan horse' effect) at the site of infection.[216,217] While two-photon microscopy allows penetration depths of around 700 um, three-photon (3P) microscopy can reach beyond 1 mm. An important challenge of IVM at depth is scattering, resulting in low SNRs, limiting visualisation of fine processes. Deep3P couples 3P microscopy with adaptive optics and DL to image deep layers of the brain without the need of a grin lens which causes physical injury at those depths. This has great potential for studying processes occurring at depths deeper than those at which we have so far visualised.
- **DELiVR**:[279] A virtual reality-trained DL pipeline for phenotype characterisation in optically cleared mouse brains imaged with lightsheet microscopy. VR was used for careful annotation of the data, and a DL was trained to recognise specific cell types and map them automatically to the Allen Brain Atlas. We consider this would be a powerful tool for the characterisation of multiple

factors governing host–parasite interactions, including cell populations, vascular damage, spatial distribution of parasites relative to tissue-specific landmarks, among others.

There are many other aspects of host–parasite interactions, and countless AI-based resources developed in other fields. Here we have just exemplified a few of the most recent.

### 3.3.3 | Understanding parasite motility

Motility characterisation has proven to be of key importance for our understanding of motile forms of Apicomplexans, and free-swimming forms of Kinetoplastids. While our initial knowledge arose from motility characterisation of parasites in vitro, more recent work has shown that motility is heavily affected by the complex environments that the parasite encounters within the host. Tracking is fundamental to understand parasite behaviour in such environments.[280–282] However, manual tracking is prohibitive in many settings and datasets due to spatial and temporal discontinuities.

Several DL methods have been generated to overcome manual tracking challenges. Below, we highlight three tools we consider relevant. We otherwise refer readers to a recent analysis by Maska et al.[283] covering a decade of developments arising from the Cell Tracking Challenge and to the work of Chai et al. discussing opportunities and challenges for DL in cell dynamics research.[284]

- **3DeeCellTracker**[285] was designed to track different cell types in different organisms (worms, zebrafish and tumour spheroids) imaged with different microscopy methods. The model performed well even in challenging conditions including large movements, cross-layer movements and weak intensities or photobleaching.
- **DeepSea**[286] allows tracking of single cells in sequences of phase-contrast live microscopy images. It was useful to monitor multiple cellular phenotypes and several cell division cycles, enabling the generation of lineage tree structures of cells. Both are major topics of interest across parasite fields.
- Other **user-friendly tools such as TrackMate** have also allowed for the incorporation of DL[287] for cell tracking.

### 3.3.4 | Understanding rare events

The idea of a smart microscope is one that can make decisions about what, when and how to image a sample autonomously. Several recent commentaries share different opinions on this topic. Some suggest that microscope design should focus on intelligent feedback guiding control, acquisition and processing, as opposed to manual operation. An example of this would be an AI-driven microscope that could be capable of adapting to the sample, only acquiring information essential to the research question[288] and using language-guided image analysis to customise workflows.[289] Micropilot[290] and the work by Mahecic et al.[291] who developed an event-driven acquisition framework, are examples of tools to facilitate this model.

Smart microscopes combined with DL could accelerate our understanding of parasitology. This would streamline data acquisition and analysis of processes including parasite interactions with the blood vasculature, parasite lineage tracing and cell cycle progression, subcellular component identification, and drug target characterisation.

## 3.4 | Bridging gaps in AI knowledge integration and fostering expertise exchange

Additional to the challenges we have discussed above, two important aspects to consider moving forward with AI are accessibility and reproducibility (Figure 8B).

### 3.4.1 | Accessibility

Image analysis lies at the intersection of several disciplines including computer science, statistics, and the field relevant to what is being imaged—in this case, biology. Knowledge exchange between experts from different disciplines is often hindered by field-specific 'jargon', and building a novel skillset such as programming can be daunting and time-consuming. For someone with little prior experience, it might also be difficult to know where to start, or even where to look for advice. Four ways to improve accessibility that we think would be beneficial for the parasitology field are:

- **Building expertise**: For scientists who have never explored image analysis or AI tools, even knowing where to look for resources might be challenging. Increasing awareness about available training programs where relevant AI-driven microscopy is taught and creating training that specifically addresses issues relevant to imaging parasitology would be beneficial. We refer readers to recent work focusing on experiences and best for image analysis workshops.[292] In addition to this, a recent community survey conducted by Sivagurunathan

et al.[293] explored views on how to bridge the gap between imaging scientists and image analysis. In their findings, they identified the need for documentation and tutorials. For current opportunities, we refer readers to curated databases, such as MicroscopyDB[294] on courses and events where further information can be found.

- **Harnessing the power of existing centralised expertise**: Harnessing the power of bioimage analysts and imaging core facilities is an important strategy moving forward. Their focus is to (a) constantly survey tool availability (b) optimise tools for specific tasks required by the research community, (c) design custom-made workflows and tools specific for the task required by the community. Linking back to building expertise, in recent years, postdoctoral programs worldwide and specialised training for the imaging and image analysis career have been designed.[295–297] Of note, a redefinition and increased recognition of the value of the imaging and image analyst specialist will only help strengthen research.[295]
- **Building international and interdisciplinary collaborations**. The international image analysis community offers significant expertise in various ways, including hackathons (e.g. AI4Life), forums (e.g. Image.sc Forum), fellowships that allow collaborative visits for exchange of experience (e.g. BINA, LABI, Global Bioimaging) and bioimaging communities that can help strengthen AI knowledge. We encourage parasitologists to engage in these opportunities.
- **Building open and user-friendly tools**. Generating tools that help bridge the gap between computational and biomedical scientists, including user friendly software and user interfaces is critical to bridge the gap between the wet lab and dry lab scientists. Making sure that resources are available in different languages also ensures inclusion, which is particularly important for public health and neglected disease control. CellProfiler,[298,299] ImageJ/Fiji[300,301] and BIAFLOWS[302] are excellent examples of this achievement. Specific tools aimed at democratic, reproducible, and collaborative access are ZeroCostDL4Mic,[303] ImJoy,[304] DeepCellKiosk,[305] CellTracksColab,[306] and DL4MicEverywhere,[307] and RENOIR[308] among others.

### 3.4.2 | Reproducibility

The reproducibility crisis is a shared concern in scientific disciplines. Already almost a decade ago, a global survey on reproducibility shared insights on key points to address.[309] Among them was selective reporting, pressure to publish, and methods and code being unavailable. These are especially prevalent problems in imaging science.[310] The increased use of AI has raised concerns about rigor and reproducibility. This includes the risks of ill-informed use of a 'black box' resulting in inadequate conclusions.[311]

Whether or not AI is used, for imaging data to be transferable, it should follow specific standardised formats and principles such as Findable, Accessible, Interoperable, Reusable (FAIR).[312] Findable imaging data should be properly identified (e.g. by cell type and imaging technique). Metadata should include information about sample treatments and imaging parameters, and it should easily searchable in a database. Unfortunately, most data that is generated does not adhere to these standards. We refer readers to existing guidelines of how to ensure accurate reporting,[313–315] and we highlight the efforts of communities such as QUAREP-LiMi in establishing guidelines to ensure reproducibility.[316,317]

## 4 | CONCLUSIONS

Parasitologists have done an excellent job at generating AI-powered tools for diagnosis. Outside of the clinical setting, parasitology research needs to fully leverage AI to maximise insights into the basic biology of these parasites and their interactions with their hosts. Altogether, the implementation of AI-powered microscopy will contribute to automating tasks to re-direct valuable human resources to other areas vital for disease control. Given the great overlap of this field with other biomedical areas, we also envisage that AI will be central to foster integrated and collaborative science in the future. Building robust AI-powered microscopy capacities worldwide and integrating interdisciplinary information with other One Health approaches will be key for better disease control and preparedness.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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