



Artificial intelligence for high content imaging in drug discovery

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Abstract

Artificial intelligence (AI) and high-content imaging (HCI) are contributing to advancements in drug discovery, propelled by the recent progress in deep neural networks. This review highlights AI's role in analysis of HCI data from fixed and live-cell imaging, enabling novel label-free and multi-channel fluorescent screening methods, and improving compound profiling. HCI experiments are rapid and cost-effective, facilitating large data set accumulation for AI model training. However, the success of AI in drug discovery also depends on high-quality data, reproducible experiments, and robust validation to ensure model performance. Despite challenges like the need for annotated compounds and managing vast image data, AI's potential in phenotypic screening and drug profiling is significant. Future improvements in AI, including increased interpretability and integration of multiple modalities, are expected to solidify AI and HCI's role in drug discovery.

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Introduction

The adoption of AI in drug discovery has been transformative, leveraging algorithms to analyze complex biological data and predict drug efficacy and safety profiles [1]. Machine learning, and lately deep learning, has contributed to these advancements by enabling neural networks to uncover hidden patterns and insights from both structured and unstructured data sources [2]. More recently, generative AI (GenAI) has emerged as a powerful methodology, for example to generate novel

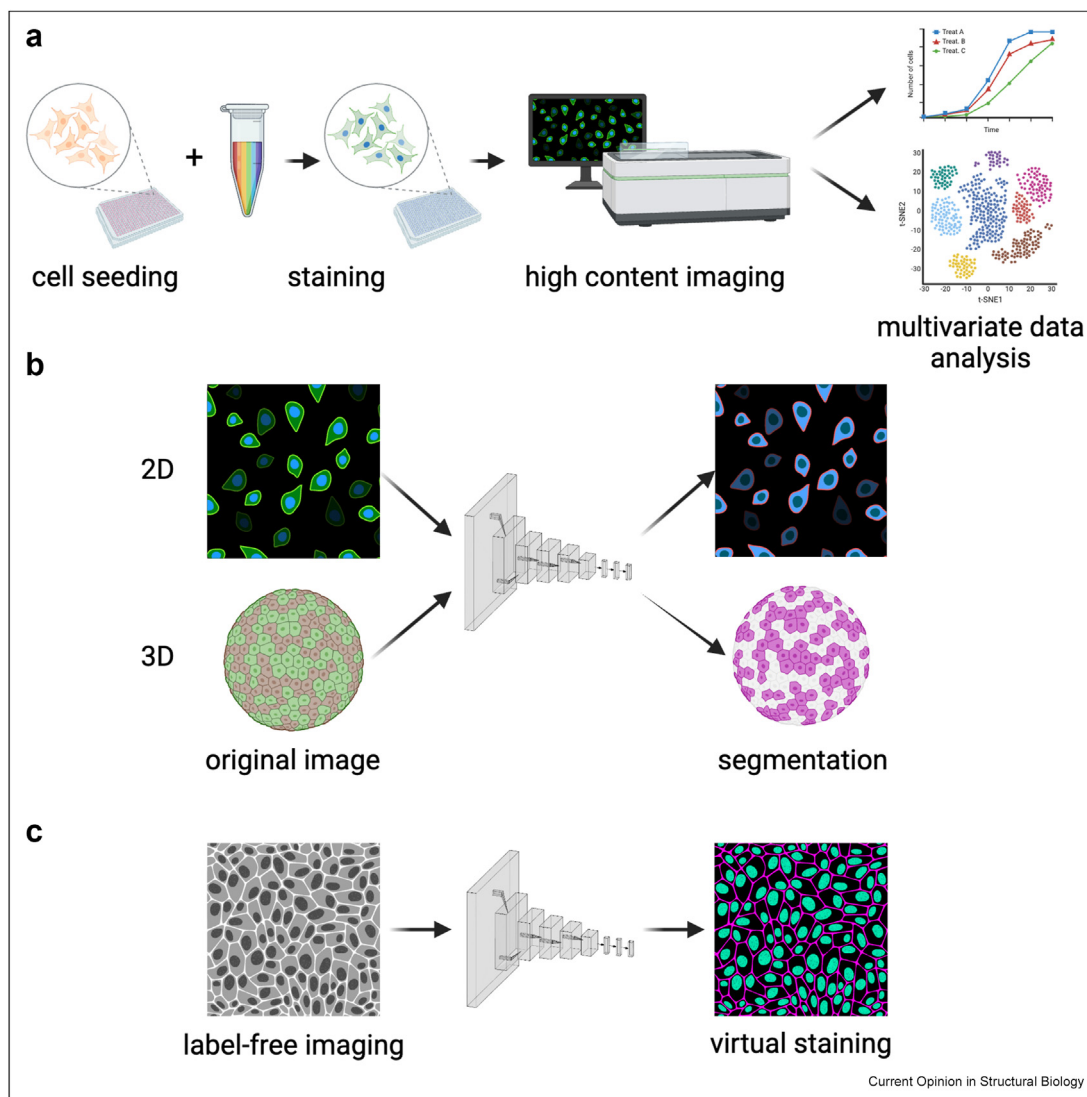
chemical entities with desired properties through their ability to learn underlying molecular representations [3]. Additionally, reinforcement learning techniques are being used to optimize lead compounds by iteratively improving molecular structures based on desired criteria such as target binding affinity or pharmacokinetic properties [4]. These and other emerging AI-driven approaches have significantly reduced the time and cost associated with traditional drug discovery methods [1] by enabling rapid screening of vast chemical libraries *in silico*, while identifying promising candidates for further experimental validation at a steadily increasing pace [5].

In the fields of imaging and microscopy, AI has revolutionized the analysis of biological images. Machine learning methods on images have traditionally utilized classical image analysis algorithms for segmentation and measurements, features have commonly been selected and designed by humans, and machine learning models such as SVM and Random Forest applied to the produced tabular datasets. When Convolutional Neural Networks (CNNs) emerged they took image analysis to the next level, for example by improving segmentation [6], feature extraction and classification [7,8]. One important characteristic of these methods is that representations are learned, and the process can be entirely data-driven. Further, the learned feature representation can in many cases capture novel aspects of the data that were not represented in manually constructed features, leading to improved predictive performance. Convolutional encoder-decoders such as the U-Net architecture, designed specifically for medical image segmentation, have set a new standard by enabling precise delineation of cellular and subcellular structures in images, even in the presence of noise and variability [9]. Moreover, deep learning models have been developed to enhance the resolution and quality of images obtained from lower-quality inputs, employing techniques such as deconvolution and super-resolution [10]. These advancements have facilitated the extraction of accurate quantitative data from biological images, supporting high-throughput analyses and the identification of subtle phenotypic changes in response to drug treatments [11].

High content imaging

High Content Imaging (HCI) combines automated microscopy with sophisticated image analysis to

Figure 1



AI-assisted high content imaging. **a**) Schematic overview of an HCI experiment. In brief, cells are seeded in a multiwell plate prior to genetic or chemical perturbation (this order can also be reversed, where cells are seeded on top of a library of chemicals or genetic perturbants). Cells are then stained using a single marker or in a multiplexed fashion. Subsequently, an automated high-throughput microscope is used to acquire multiple images of the samples, which are then analyzed to extract multivariate data. **b**) Example of a CNN used for segmenting cells in both 2D and/or 3D images of cellular samples. **c**) A CNN is used to predict the fluorescent stain of a label-free (typically brightfield) image, which is known as virtual staining. Created with BioRender.com.

examine cells in a high-throughput manner (Figure 1a). This technology is characterized by its ability to capture thousands of images, allowing for the comprehensive assessment of compound effects on cellular phenotypes upon a subsequent analysis [12]. The use of AI, particularly in the form of deep learning, has enhanced HCI by improving image quality, segmentation of objects, and feature extraction, enabling the identification of complex phenotypic patterns that correlate with disease states or drug efficacy [11]. The use of automated microscopes equipped with imaging modalities such as fluorescence and confocal microscopy, alongside

AI-driven image analysis software, has dramatically increased the throughput and accuracy of phenotypic assays [13].

In HCI, the size and resolution of images is not massive (in contrast to e.g. whole-slide imaging of tissue sections) but instead a larger number of images is generally produced. While traditional quantitative methods in many cases relied on segmenting objects (e.g. cells) and extraction of pre-defined features, AI methods taking images as input can apart from segmented objects be applied to the entire image and image crops. At the same

time, AI-based methods for cell segmentation are continuously improving [14], including the development of foundation models that are pre-trained on large amounts of data [15]. Improved cell segmentation is early in analysis pipelines, and hence has an important and direct effect on all downstream analysis steps.

AI in fixed sample imaging

The application of AI in fixed sample imaging is a rapidly evolving field that encompasses the detailed analysis of stained cells or tissues that have been fixated or immobilized. This allows for an in-depth examination of cellular morphology and the distribution of specific biomarkers, providing valuable insights into various biological processes [16]. Deep learning models have shown remarkable proficiency in classifying cells based on subtle morphological changes [14], identifying nuanced differences in staining patterns, and for instance, accurately quantifying the expression levels of target proteins within these samples. These advanced capabilities hold significant importance for high-throughput screening campaigns where there is a need to swiftly assess the effects of thousands of compounds [17]. The ability to rapidly and accurately analyze large volumes of image data can greatly expedite research efforts aimed at understanding complex cellular interactions and uncovering potential therapeutic targets [18].

AI in live-cell imaging

Live-cell imaging is a critical technique for observing dynamic cellular processes without compromising cell viability, presenting unique challenges in tracking temporal cellular dynamics [19,20]. Recent advancements have seen the development of AI models that significantly enhance the capability to automatically monitor cell movement, division, and morphological changes in real-time, thereby providing in-depth insights into drug action kinetics [21]. Long Short Term Memory (LSTM) and Convolutional LSTM Networks have been shown to be powerful for analyzing live-cell images [22], but more recently attention-based Transformers have become the most widely used methodology for analyzing live-cell imaging [23].

The integration of time-lapse imaging with AI analysis facilitates a nuanced examination of drug effects on biological processes such as cell cycle, migration or apoptosis, offering a dynamic and comprehensive perspective on cellular responses to pharmacological treatments [24]. This AI-enhanced approach to live-cell imaging allows for a detailed analysis of the temporal and spatial dimensions of drug-cell interactions, incorporating an additional layer of information compared to fixed sample imaging. By enabling precise tracking of cellular dynamics, researchers can uncover the mechanisms underlying drug efficacy and toxicity, which could

be used to potentially aid therapeutic interventions [25]. HCI with live-cell imaging however comes with additional challenges in terms of the vast amounts of data generated, necessitating substantial computational infrastructure and analysis pipelines.

AI in 3D imaging

The use of AI in three-dimensional (3D) imaging has revolutionized the reconstruction and analysis of complex tissue architectures and organoids [26]. Deep learning, particularly CNNs, play a crucial role in segmenting and analyzing 3D structures, enhancing our ability to visualize the effects of drugs on tissue morphology and function (Figure 1b) [27]. These advancements have the potential to aid the development of 3D cell culture models, thereby offering a physiologically relevant platform for drug screening and efficacy assessment [28]. CNNs, optimized for grid-like data structures such as images, enable detailed examination of tissue and organoid structures, facilitating quantitative analysis of tissue responses to pharmacological interventions [29]. This capability is essential for phenotypic drug discovery, allowing for high-throughput screening of compounds in models that closely resemble human tissue complexity. Despite challenges such as computational demands and algorithmic development for complex data sets, advancements in computational power and algorithm efficiency are mitigating these issues, broadening the accessibility and application of AI in biomedical research [30]. In essence, AI-enhanced 3D (and also 2D) imaging is an important component in today's drug discovery, offering in-depth insights into disease mechanisms and therapeutic interactions within physiologically relevant models [31].

Application: Drug screening

Label-free screening

The integration of AI in label-free HCI, as referred to the use of e.g. brightfield images without the need for a fluorescent stain or dye, for phenotypic screening represents a paradigm shift in drug discovery methodologies [32,33]. This combination harnesses the power of AI to interpret complex biological data obtained from label-free HCI, a technique that allows for the observation of cellular and tissue phenotypes in their most native and undisturbed state (Figure 1c). AI algorithms offer sophisticated means for analyzing the intricate patterns and dynamics captured in label-free images, facilitating a deeper understanding of drug effects on cellular phenotypes [34,35,33]. Deep learning models, such as CNNs, have been at the forefront of these advances, demonstrating an unparalleled ability to extract and learn features from label-free imaging data. These models can identify phenotypic alterations indicative of biological responses to drug treatments, which might be imperceptible to human observers or traditional image analysis techniques [36,37]. However, the application of

AI in this context is not without its challenges. One of the primary concerns is the requirement for extensive, annotated datasets to train these sophisticated models. The generation of such datasets in label-free HCI is resource-intensive and requires careful experimental design to ensure the capture of relevant biological variability [38]. Moreover, the interpretability of DL models remains a significant hurdle. The complexity and opacity of these models often make it difficult to understand the basis of their predictions, posing challenges for biological validation and hypothesis generation [39]. More recently, Generative AI has been used to predict virtual stains from label-free imaging (Figure 1c) [40], or for direct classification of MoAs [33], and large datasets for AI are emerging [41]. As there are significant benefits in time, cost, and toxicity when using label-free imaging compared to staining with fluorescent dyes, the advances in AI methodologies makes label-free HCI a technology with high potential to be increasingly used in drug discovery.

Single marker screening

Single marker HCI, a traditional method of using a single dye, antibody or fluorophore to label and visualize a specific target or component within a sample, is a pivotal technique in cellular biology and drug discovery [42]. Single marker imaging offers precise visualization and quantification of cellular components or activities through fluorescence-based assays [43]. Utilizing a single fluorescent dye to target specific cellular features, this method illuminates complex biological processes and evaluates the impact of compounds on them [44]. It is of special value in large scale screening efforts aiming to quickly gauge the effects of numerous potential drugs on cellular pathways, thus streamlining the identification of viable drug candidates. AI has significantly advanced single-dye phenotypic HCI by automating the analysis of large image datasets with remarkable speed and accuracy. Deep learning-based AI algorithms are particularly effective in identifying and quantifying changes in a given marker of interest used to identify a phenotype of interest. This AI-enhanced approach is key in pinpointing inhibitors or activators of specific proteins, markedly boosting the efficiency and precision of the drug discovery process [45]. AI's role extends to enhancing the throughput and reliability of phenotypic screening by automating the detection of subtle fluorescence changes, indicative of protein activity changes. This automation facilitates the rapid screening of extensive compound libraries, improving the selection accuracy for candidates with the best therapeutic potential [46]. Moreover, AI's integration into phenotypic screening reflects a broader shift towards using computational tools for analyzing complex biological data, a relevant example being how machine learning can classify cellular images based on phenotypic changes and identify patterns correlating with genetic or chemical perturbations [11].

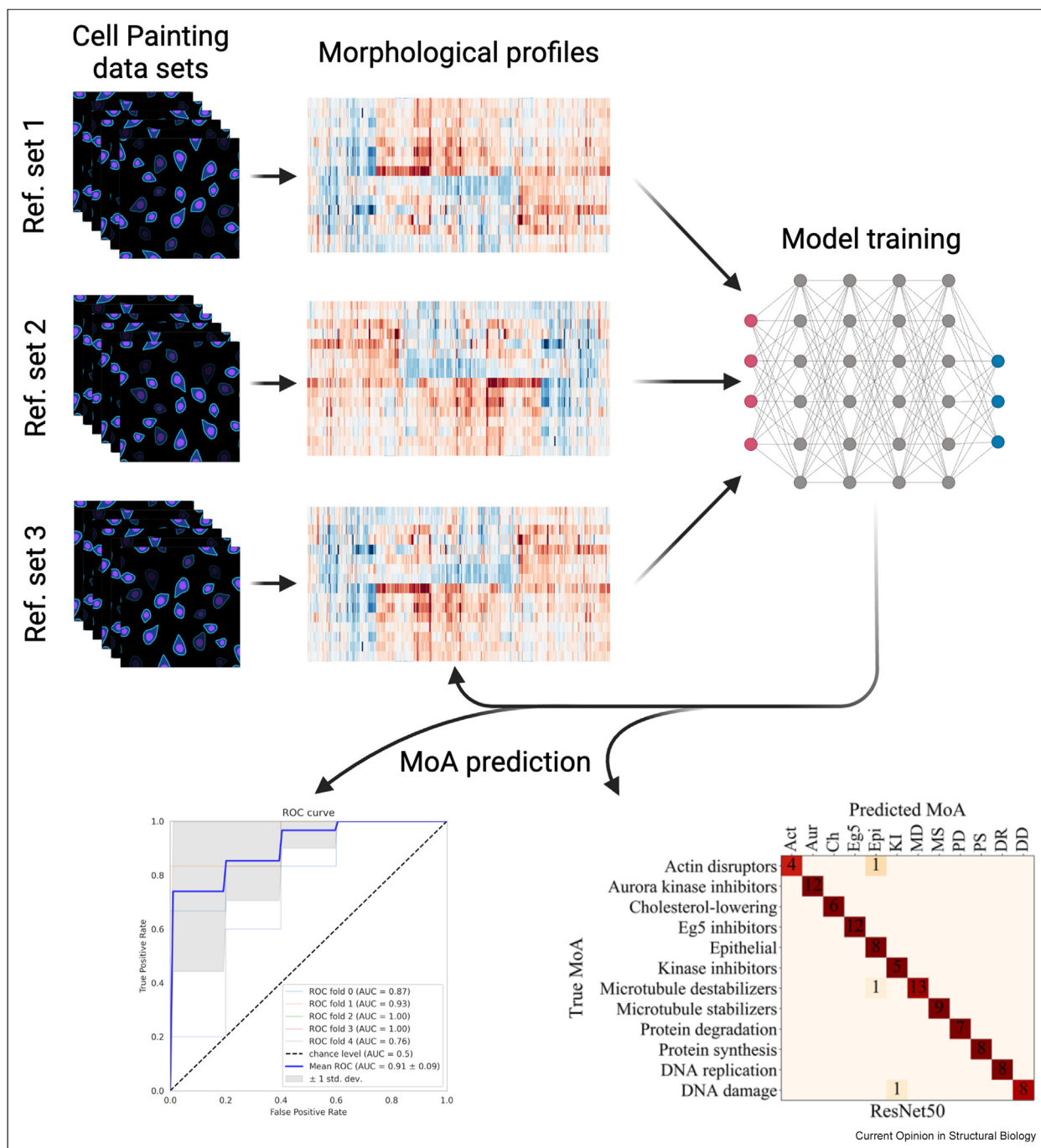
Screening with multiplexed dyes

AI has emerged as a transformative force in the realm of drug discovery, particularly through its application in multiplexed HCI for phenotypic screening. At the heart of this evolution is the Cell Painting technique, a high-throughput, richly informative method that captures a comprehensive cellular phenotype by staining cells with multiple fluorescent dyes [47]. Using automated microscopy, this approach can generate large datasets, capturing nuanced details of cellular states and responses to drugs, which are inherently amenable to AI-driven analyses [47,48]. Traditional image analysis of this type of data relied on human-derived feature extraction, e.g. using the Cell Profiler software [49]. The application of AI to multiplexed HCI data facilitates the data-driven extraction of feature embeddings that can be used to detect patterns and relationships that would be otherwise imperceptible. These algorithms can identify subtle phenotypic changes, predict compound toxicity, and elucidate mechanisms of action, thereby accelerating the identification of therapeutic candidates [50–52]. However, the sheer volume and complexity of the data demand substantial computational resources and sophisticated algorithms that can accurately process and interpret the information without succumbing to overfitting or bias [53]. More recently, self-supervised transformer methods have emerged that are pre-trained on substantial image-based datasets to provide efficient learned representations, such as for single-cell morphology [54]. These methods have their origin in other scientific domains e.g. NLP and computer vision, and have the potential to further improve accuracy of AI-based analysis on cell morphology data. This constitutes a step in the direction towards foundation models that are useful in zero-shot learning settings where no fine-tuning to specific problems is required [55].

Application: Compound profiling

Compound profiling, including the assessment of the compound's mechanism of action (MoA) and potential bioactivity is an important challenge in drug discovery, not the least in safety assessment [56]. AI in the form of supervised learning is the most widely used approach to this end. This methodology relies on establishing reference datasets with compounds having measured endpoints (often referred to as 'ground truth') and then training a machine learning model to predict the outcome of new compounds (Figure 2). The most common approach has been cheminformatics approaches to train models based on features derived from chemical structure (structure-activity relationships or QSAR). AI models trained on data from HCI here represents a new avenue, with potential to overcome the inherent limitation of cheminformatics models constrained by the small fraction of chemical space covered by the training data. To this end, AI models trained on HCI represent a transformative approach and have shown considerable promise in interpreting the

Figure 2



Compound profiling using supervised learning for MoA prediction. Compounds having known MoA are used to establish reference datasets. Features can be extracted from images to make up morphological profiles, it is common to use mean or median cells for a specific perturbation. The datasets constructed in this way can be used to train machine learning models that can be validated using cross-validation to assess e.g. accuracy or ROC-curves, and ultimately be used to predict MoA's of a novel compound. Created with BioRender.com.

complex, multidimensional data generated by HCI [57] and with applications for assessing compound MoA and bioactivity [58], and evaluating safety profiles [56]. The utility of machine learning in classifying compounds

based on phenotypic outcomes, extensively covered by Caicedo, Scheeder and colleagues [59,60], highlights the potential for AI to streamline the identification of therapeutic candidates with desired biological activities.

Machine learning and compound predictions

Image features extracted using e.g. Cell Profiler together with tabular machine learning methods such as SVM, Random Forest and XGBoost have been used with great success [59]. Such features offer means for interpreting results via feature importance methods, and Seal et al. integrated these features with data from cell health assays to improve interpretability [61].

Deep learning algorithms such as CNNs and Recurrent Neural Networks (RNNs) significantly enhance the analysis of HCI data. CNNs have proven effective in classifying complex phenotypes indicative of specific MoAs, as highlighted by Godinez et al. [27], who showcased CNNs' ability to discern intricate patterns in cellular images. RNNs, while less directly applied to HCI, offer valuable insights into the temporal dynamics of perturbation effects, analyzing sequential changes in cellular behavior over time [62]. Learned representations of image-based profiling are now widely used in the scientific community [8].

The prediction of compound bioactivity and safety through supervised learning leverages the high-throughput, quantitative nature of HCI. By correlating specific phenotypic changes with known bioactivity or toxicity profiles, AI models can forecast the effects of new compounds, potentially reducing the reliance on traditional, more resource-intensive methods. In summary, machine learning models can accurately predict the toxicity of compounds by analyzing cellular features captured through HCI, complemented with chemical data, underscoring the role of AI in enhancing the safety assessment of drug candidates [63].

Need for reference data

At the heart of supervised learning is the critical need for extensive, well-annotated reference datasets. The efficacy of AI-driven methodologies in drug profiling and the subsequent identification of compound effects is fundamentally contingent upon the availability of such datasets [64], such as accurately predicting phenotypic outcomes and MoA based on high-dimensional imaging data from HCI [65].

The creation and curation of comprehensive databases that meticulously catalog phenotypic responses to a wide array of compounds with known endpoints are paramount. Such databases are invaluable not only for training AI models with high precision and reliability but also for facilitating a deeper understanding of compound efficacy, toxicity, and potential off-target effects. The efforts carried out by the Joint Undertaking in Morphological Profiling (JUMP) Cell Painting consortium [66], were aimed at precisely this, generating large high-quality morphological reference datasets, and making them freely available for their use to, for example, train AI models.

The reliance on large, well-annotated reference datasets underscores a broader challenge within the field: the necessity for high-quality data annotation and the establishment of standardized protocols for data collection and analysis [67]. The heterogeneity of data sources and experimental conditions can introduce variability that complicates model training and validation. As such, efforts to harmonize data standards and improve the quality of compound annotations are critical for enhancing the robustness and applicability of AI models based on HCI data.

AI model life cycle

The AI model life cycle in the HCI domain involves several critical stages: data collection, preprocessing, model training, validation, and deployment. Each of these stages plays a vital role in the overall success of AI applications in drug discovery.

Data collection is the foundational step, and there is no AI architecture that can compensate for low quality or lack of sufficient data in the training. For HCI, this necessitates that images not only possess high resolution but also exhibit minimal noise and maintain consistent staining quality across datasets. These criteria are essential for capturing the nuanced biological responses to pharmacological interventions, thereby enabling the accurate identification of potential therapeutic targets and compounds. The complexity and heterogeneity of biological data require meticulous data management practices to ensure the integrity and usability of the captured data [68,69].

Data preprocessing

Data preprocessing techniques play a crucial role, serving to refine and prepare imaging data for AI analysis. Techniques such as normalization, augmentation, and denoising are employed to mitigate the effects of variability in image quality and ensure uniformity across the dataset [48]. Normalization adjusts the intensity values across images, facilitating a more consistent basis for comparison, while augmentation techniques, such as rotation, flipping, and scaling, artificially expand the dataset, thereby enhancing the robustness of the AI model against overfitting by providing a more comprehensive representation of possible variations. Denoising, on the other hand, is critical for removing artifacts and random noise from images, which, if left unaddressed, could lead to misleading interpretations and inaccurate model predictions [70,71]. The preprocessing of data not only enhances the quality of the input fed into AI models but also significantly impacts the efficiency of model training and the accuracy of subsequent predictions. High-quality, well-preprocessed data can dramatically improve the learning process, enabling the development of models that are both more sophisticated and capable of generating reliable, reproducible predictions.

Experimental design is an often overlooked topic in terms of data quality, as how the experiment is carried out in terms of samples, batches, replicates etc can have a great effect on the resulting data. For HCI where automated microscopes and samples in microplates are used, the placement of samples, controls within and across plates can have profound impact. Using AI to design effective microplates is one approach that has shown to reduce errors in subsequent data normalization [72]. Batch effects can substantially limit the integration and comparison of data within but more importantly between labs. Variation in protocols and equipment can contribute to this end. Apart from well-designed experiments, it is also important to apply adequate batch correction techniques [73].

Model training and validation

Model training is at the heart of the AI model life cycle, and adequate validation methodologies are crucial in order to reduce the chance of overfitting and ensure reliability, accuracy, and generalizability to real-world situations [74]. Cross-validation, a technique that involves partitioning a dataset into complementary subsets, performing the analysis on one subset (the training set), and validating the analysis on the other subset (the validation set), is widely used for assessing the model's predictive performance and generalizability [75]. Regularization methods, such as L1 and L2 regularization, further contribute to the model's robustness by penalizing complexity and thereby preventing overfitting, ensuring that models remain generalizable across diverse datasets [76]. Ensemble learning, which combines multiple models to improve the overall performance, has been shown to significantly enhance the robustness and accuracy of predictive models in drug discovery applications [77].

Transparency in AI modeling facilitates a deeper understanding and trust in AI-driven decisions, which is critical for their acceptance and implementation in the drug discovery pipeline. Interpretability, the degree to which a human can understand the cause of a decision made by an AI model, is also important for validating the model's predictions and for the scientific community's acceptance of AI as a tool in drug discovery [78]. For HCI, human inspection is less prominent due to the sheer number of produced images, but still serves as an important tool when analyzing data and troubleshooting the training of deep learning models [79].

Model deployment

Deployment of AI models into the drug discovery workflow represents the culmination of the model life cycle, where models are made available to be used for e.g. compound screening, predicting activities, and identifying potential off-target effects. This stage requires seamless integration of AI tools with existing

bioinformatics and cheminformatics systems, necessitating robust software development practices and interdisciplinary collaboration [68,69]. The adoption of best practices in data management, model development, and validation is imperative to ensure that AI models can be relied upon in decision making. This necessitates a collaborative effort among biologists, data scientists, and software developers, fostering an interdisciplinary approach that is critical for the development of effective workflows. Using cloud computing and MLOps methodologies have proven to be effective to realize these goals [80]. Such processes streamline AI development and ensure that models can be continuously deployed and predictions be made using models trained on the latest available data.

Conclusions and outlook

The integration of AI with HCI represents a big shift towards data-rich drug discovery. With the increasing availability of large image resources and technologies for rapid and cost-effective data generation, the combination is fueling innovative methodologies for high-throughput screening, detailed phenotypic profiling, and effective elucidation of drug mechanisms of action. AI-driven approaches have demonstrated the potential to decipher complex biological datasets, enabling the identification of novel therapeutic targets and biomarkers with unprecedented speed and precision [81,82].

Despite these significant advancements, the field faces persistent challenges that must be addressed to fully harness the power of AI in drug discovery. One of the primary concerns is the quality of data, which directly impacts the performance and reliability of AI models. Inaccuracies, biases, and inconsistencies in datasets can lead to misleading conclusions, underscoring the need for high-quality, well-annotated reference datasets [68]. Furthermore, the reproducibility of AI models remains a critical issue, with variations in experimental conditions and data processing often leading to discrepancies in results across studies [83]. Another challenge lies in managing the large amounts of data in the form of images that is produced via automated microscopy. When going from fixed to live imaging, this becomes even more challenging.

Interpretability of AI models also poses a significant challenge, particularly in the context of HCI where the complexity of biological systems and the mechanisms underlying drug effects are often not fully understood. The development of more transparent and interpretable AI models is crucial for gaining trust and understanding of AI-driven discoveries among the scientific community [84].

The field of AI is advancing at a high pace, and new methodologies and architectures are continuously

emerging from different fields where imaging is the main data source, with computer vision being a driving force. This trend is likely to continue and will surely propel new opportunities when analyzing HCI data within a drug discovery context.

The potential of AI to transform drug discovery is immense, and HCI constitutes an experimental modality that is cost-effective and readily available. As these technologies continue to evolve and integrate, they hold high potential to contribute to an accelerated discovery of novel therapeutics.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to improve language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Declaration of competing interest

OS and JCP declare ownership in Phenaros Pharmaceuticals AB, a company exploiting AI, automation and HCI for drug discovery.

Data availability

No data was used for the research described in the article.

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