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Current Opinion in Chemical Biology

Image-based cell phenotyping with deep learning

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Abstract

A cell's phenotype is the culmination of several cellular processes through a complex network of molecular interactions that ultimately result in a unique morphological signature. Visual cell phenotyping is the characterization and quantification of these observable cellular traits in images. Recently, cellular phenotyping has undergone a massive overhaul in terms of scale, resolution, and throughput, which is attributable to advances across electronic, optical, and chemical technologies for imaging cells. Coupled with the rapid acceleration of deep learning–based computational tools, these advances have opened up new avenues for innovation across a wide variety of high-throughput cell biology applications. Here, we review applications wherein deep learning is powering the recognition, profiling, and prediction of visual phenotypes to answer important biological questions. As the complexity and scale of imaging assays increase, deep learning offers computational solutions to elucidate the details of previously unexplored cellular phenotypes.

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Current Opinion in Chemical Biology 2021, **65**:9–17

This review comes from a themed issue on **Machine Learning in Chemical Biology**

Edited by **Conor Coley** and **Xiao Wang**

For a complete overview see the Issue and the Editorial

https://doi.org/10.1016/j.cbpa.2021.04.001

Keywords

Deep learning, Cell phenotyping, Phenotypic screening, Image analysis.

Introduction

Visual phenotypic variations are everywhere in nature. All living things have structural adaptations to their environments that can be visually recognized at macroscales and microscales. Microscopy became a fundamental tool for cell biology because it overcame the limitations of the human eye and allowed us to observe structural and molecular adaptations of single cells in their microenvironments. Now, we have unprecedented access to optical, electronic, and chemical technology to image cellular structures at high spatial and temporal resolution, which is instrumental in unveiling cellular states. Furthermore, computational methods are being developed to extract the wealth of information contained in biological images [1].

Cellular phenotypes in images are inherently unstructured and irregular, and in contrast to other probes or biological assays (which deliver specific biological quantities), images are made of pixels. Thus, the main challenge is to decode meaningful biological patterns from pixel values with accuracy and sensitivity. Image processing and classical machine learning techniques have been widely used to approach this challenge [2,3]. However, these techniques have limited power to realize the full potential that visual phenotypes have for quantitative cell biology. Capturing the complexity and subtlety of cellular phenotypes in images requires high-level understanding of important visual variations, a capability that is more readily delivered by deep learning [4,5].

The development of new deep learning–based computer vision methods has enabled a new wave of image analysis techniques to flourish. Deep learning has shown tremendous promise in solving medical diagnostic tasks such as classification of radiographic and pathological clinical images [6,7], and has provided solutions to complex problems that were intractable before, such as conditional image generation [8–10]. Deep learning has also been applied to analyzing cellular imaging tasks such as finding cells in images without manual intervention [11–14] and even powering industrial-level drug discovery wherein thousands of cellular phenotypes need to be characterized [15,16]. The success of these applications highlights the rich potential of imaging coupled with efficient deep learning for capturing and quantifying meaningful biological activity in a wide range of problems.

In this article, we use the term 'visual phenotyping' — or image-based cell phenotyping — to describe the process of understanding relevant cellular traits in a biological experiment through image analysis. This broad definition opens the spectrum of image analysis techniques to novel approaches that harness any important characteristic of cellular phenotypes for advancing cell biology. Here, we survey cell biology

Box 1. Virtual screening of compound activity.

Virtual screening aims to predict the outcome of a high-throughput biological assay to test the cell's response to chemical perturbations. Traditional compound screens rely on specific molecular tests, on specialized biochemical/functional assays, or on cell-based assays with markers of specific activity such as proteins of interest, DNA damage, or cell proliferation. In contrast, virtual screening with machine learning can use image features of cell morphology from a generic imaging assay (such as Cell Painting) to predict several of these assay outcomes simultaneously without physically running them. This strategy decreases the number of experiments needed to test an entire compound library with hundreds of assays [94–97], resulting in improved hit rates and accelerating the pace of drug discovery [95].

Methods for virtual screening of compounds usually follow an image-based profiling approach, wherein the response of a population of cells is first characterized and aggregated for each compound (Profiling cell state), followed by supervised machine learning using ground truth data obtained for a few example compounds using specialized assays (Recognizing phenotypic traits of cells). This strategy allows researchers to perform virtual screening using image-based profiles in combination with chemical structures and gene expression [96,98] and also to extend it to other applications such as predicting cell health phenotypes in Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). based perturbations of cancer cell lines [97].

Deep learning has been explored for virtual screening using end-to-end neural networks trained to predict assays directly from images, demonstrating higher accuracy than using precomputed image-based profiles [94]. In addition, modeling the relationships between visual phenotypes and chemical structures using deep learning is an emerging research field that can complement assay prediction in virtual screens. For example, the generation of molecules that would produce desired morphological changes has been explored for de novo hit design [99], and in the opposite direction, generative models have been trained to predict morphological changes, given a specific molecule [100]. These advances in compound activity prediction are potentially transformative for drug discovery research and will likely continue to evolve with better models and more data.

problems and applications wherein deep learning applied to images plays a central role. We review advances in visual phenotyping in cell classification (Recognizing phenotypic traits of cells) and cell segmentation (Box 1) and also consider new approaches that are expanding our capacity to drive biological discovery, including representation learning (Profiling cell state) and generative modeling (Predicting visual phenotypes). Outside this scope are many other important applications such as human tissue analysis in pathology [17,18], clinical diagnostics based on biomedical images [6,7,19], and live cell tracking [20–23]. We do not cover the mathematical foundations of deep learning [4,24] or aspects related to programming frameworks and computing infrastructure [5,25]. Our goal is to provide an overview of the problems that deep learning applied to images can solve to advance questions in basic and applied biological research.

Recognizing phenotypic traits of cells

Given a visual phenotype of interest, how do we automatically identify it in images? If such patterns can be formulated as explicit input–output relationships, supervised machine learning offers ways to train a computational model to identify the relevant visual patterns in images. The trained models can then be applied to new images to recognize and quantify the phenotypes of interest that correspond to various visual patterns.

One application of supervised machine learning for recognizing visual phenotypes in cells is to identify different proteins and their locations. Using images from the Human Protein Atlas [26], which capture the spatial distribution of proteins through immunofluorescence, several deep learning models have been successfully trained to automatically recognize and annotate protein localization patterns [27,28]. In addition, alterations in the cellular structure can be a recognizable phenotypic trait of disease, such as cancer. A deep neural network was shown to successfully classify the type of lung cancer in tissue samples, with performance similar to trained human pathologists, and it was even able to predict the mutated genes [17]. Deep learning has been found to recognize other cellular traits such as response to stress and cell age [29], as well as red blood cell quality [30,31].

Visual phenotypes change over time in response to cellular dynamics, revealing important information for understanding biological processes. For instance, Neumann et al. [32] leveraged high-throughput time-lapse imaging of single-cell chromatin morphology and machine learning to enable a genome-wide siRNA screen for genes regulating the cell cycle, yielding 12 newly validated and hundreds of novel candidate mitotic regulator genes. In a related study, a deep neural network model was trained to classify cell cycle stages using categorical labels, and the learned features reconstructed their continuous temporal relationships [33]. Notably, deep learning models have also been trained to accurately predict the future lineage trajectory of differentiating progenitor cells in culture based on time-lapse, label-free light microscopy images up to three generations earlier than what previous methods had achieved [34,35]. These examples highlight the power of identifying temporal changes in visual phenotypes automatically.

A common aspect of the applications mentioned previously is the availability of ground truth data for training supervised learning models, which are collected manually by experts or with the assistance of biological probes (Figure. 1). This opens up the opportunity to train end-to-end deep learning models that make predictions directly from image pixels without additional processing

or intervention, which is useful for automating tasks that depend on visual phenotyping and for scaling up accurate analyses. Beyond revealing basic cellular functions, supervised learning for recognizing visual phenotypes is also used to guide treatment design and drug discovery, wherein detecting specific traits is critical. A good example of this is image-based assay prediction (Box 1). Next, we discuss how to quantify visual phenotypes when no ground truth data are available for training supervised learning models.

Profiling cell state

A major innovation in visual phenotyping is 'image-based profiling,' wherein phenotypes are considered continuous rather than categorical. Building feature representations that capture cell state from images is the main goal, which enables us to match and compare phenotypes even if they are unknown ahead of time. This approach has many applications in cell biology, including characterizing chemical or genetic perturbations [16].

A standard approach to high-throughput image-based profiling of molecular perturbations (such as Cell Painting [36,37]) is as follows. The samples are prepared in a multiwell plate format and are subsequently exposed to a systematic array of individual or combinatorial perturbations. The microscopy images are then acquired after staining or 'painting' the cells and subcellular components by attaching different fluorescent tags. A key next step is to extract quantitative multidimensional features, also known as cellular 'profiles,' from each image, either at the individual-cell level or for entire fields of view [38]. These image-based features play a similar role in representing the cellular phenotype as do gene expression measurements in transcriptional profiling [39].

The last decade has seen a rapid development of computational techniques for image-based profiling [16,40]. Usually, the process involves identifying single cells in images (Box 2) and then computing their morphology features for unbiased analysis (Figure. 2). Commercial microscopy software and open-source tools such as CellProfiler [41], EBImage [42], and ImageJ [43] offer modules to segment individual cells and extract hundreds of features for each. These single-cell–level profiles can then be aggregated to obtain population-level profiles. Several strategies for computing aggregated profiles from segmented cells exist, but in practice, a simple median-based profile is known to be quite powerful [44,45]. Alternatively, some tools also offer strategies to extract population-level profiles directly from the field of view without the need for segmentation [46,47]. Similar to transcriptional profiling, image-based features are sensitive to technical variation, resulting in batch effects that can be detrimental to downstream analysis. Strategies to mitigate batch effects include techniques ranging from variation normalization [48] to training a generative model for batch equalization [9].

The central component of image-based profiling is feature representation, which allows to match and compare phenotypes using a similarity measure. There are two main strategies for computing feature representations: (1) engineered features and (2) learned features. Engineered features involve measuring the number of cells/subcellular components, classical

Box 2. Cell segmentation.

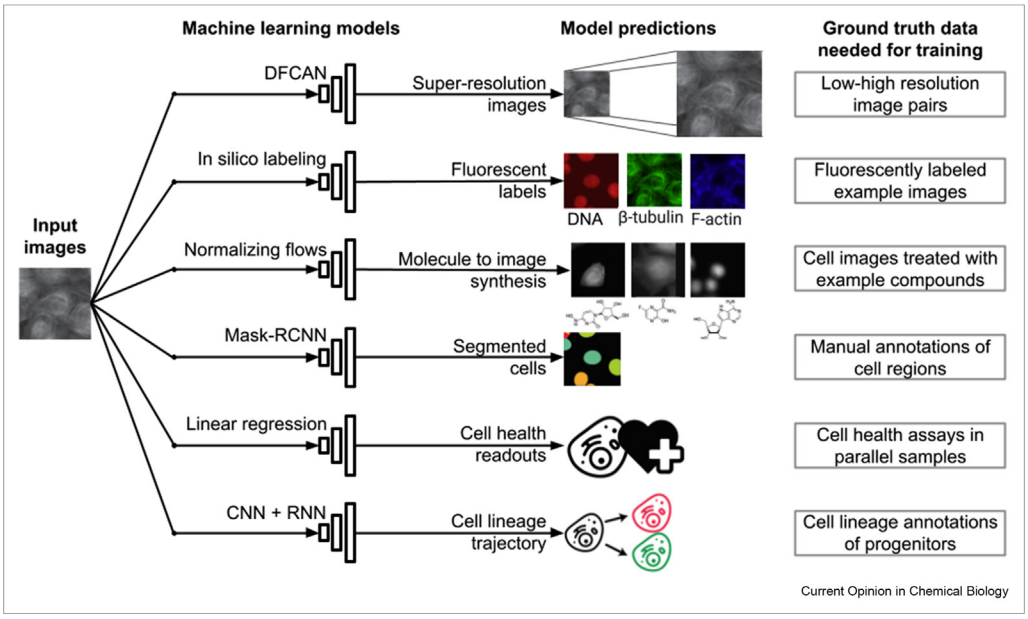
Cell segmentation, in essence, involves classifying pixels to belong to a biologically meaningful structure or the background (Fig. 2). Machine learning for cell segmentation has been available for a long time in user-friendly software such as ilastik [101,102], and deep learning is now aiming to achieve cell segmentation without any user intervention. Different types of deep learning models have been designed for cell segmentation including the U-Net model [91,92], DeepCell [103], and Mask RCNN [90,93], all of them able to achieve improved accuracy [11,104].

There are two main challenges in building new cell segmentation tools: (1) manually annotated data need to be collected for training, and (2) the training of deep learning models on those data still requires substantial human effort (and data science skills) to tune hyperparameters and to rectify faulty output. To address the first challenge, the collection of annotations for image analysis may be crowdsourced, obtaining data quality close to that of experts [105], or obtained with fluorescent labels used only to gather training data for segmenting unlabeled images [66]. To address the second challenge, new methodologies are being developed to automate neural network training, resulting in models that need little user intervention during training, while still achieving high segmentation accuracy [106].

Segmentation models can be made generic enough to identify cells across many imaging experiments [11] by collecting a representative set of manually annotated cells and training a single model once to be reused in many problems. NucleAIzer [12] and Cellpose [13] are tools that take this approach for nucleus and cytoplasm segmentation, respectively, requiring minimal user intervention. Mesmer [14], a deep learning segmentation model trained on TissueNet, the largest data set of manually annotated cells collected so far, simultaneously identifies both the cytoplasm and the nucleus in microscopy images. Another approach to reusing existing deep learning–based models is the creation of model zoos — open-access databases with user-friendly interfaces that facilitate sharing of ready-to-use trained models [107]. All these efforts are successful examples of deep learning–based tools that bring the promise of fully automated cell segmentation closer to reality.

Cell segmentation has many applications in biology, and deep learning–based models are already powering biological studies, while introducing fewer quantification errors than classical approaches [104]. Widespread adoption of such methods will depend on the availability of reliable pretrained models and user-friendly tools for everyday usage in the laboratory. Some of the open challenges in cell segmentation include the adoption of active learning to interactively annotate only the cells that the model finds challenging to segment and the creation of open collaborative data sets for training larger robust models.

Figure. 1



Example applications of supervised machine learning models on microscopy images. These models can take input bright-field images, time-lapse microscopy sequences, Cell Painting images, or precomputed image features. Models can be trained to predict a diversity of phenotypic or perturbation information, including higher resolution images [68,70], effects of compound treatments [56,89], segmentation maps [90–93], and fluorescence labels [8,10,63]. While the training process requires input/output pairs using ground truth data (Recognizing phenotypic traits of cells), trained models can predict these outputs from new images in an automated way for future analysis. DFCAN, deep Fourier channel attention network; Mask RCNN, Mask Region-based Convolutional Neural Network; CNN + RNN, convolutional neural network + recurrent neural network.

Figure. 2

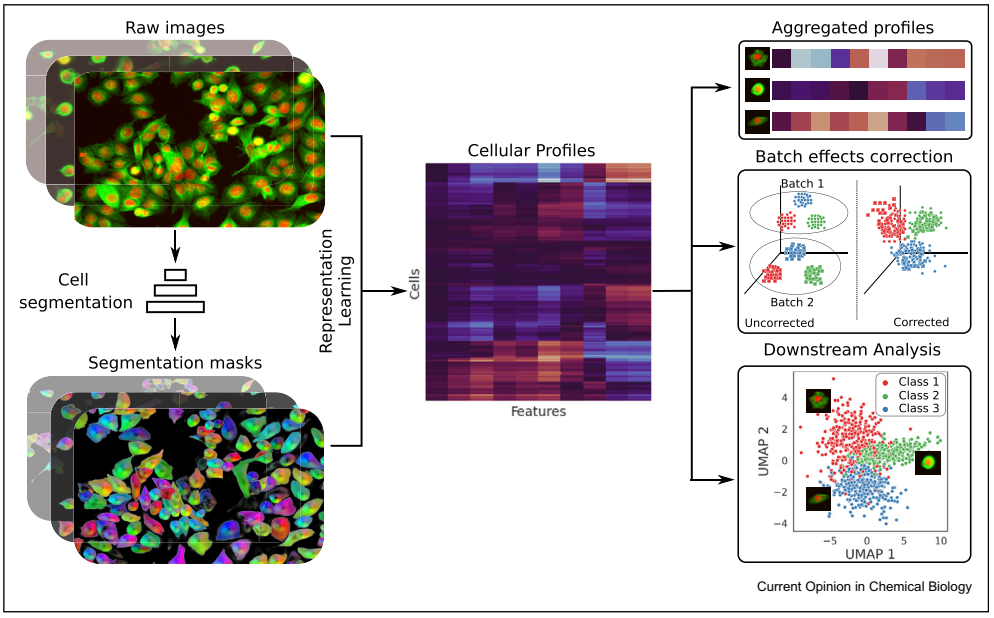


Illustration of the typical computational workflow for image-based cell profiling. Single cells are first identified using image segmentation (Box 2), and then, morphology features are computed to quantify cell state (Profiling cell state). The single-cell-level profiles can then be aggregated to obtain population-level profiles. Image-based features can then be analyzed to identify and correct potential batch effects. These profiles generate follow up experimental hypotheses and to obtain novel biological insights in the corresponding biological application.

cytometry-based features [49], and other coefficients such as Zernike shape features and Gabor texture features [36]. Depending on the type of experiment, they can also represent normalized pixel intensities of fluorescent markers after segmentation for their analysis [50]. Learned features commonly involve a deep learning model, such as a convolutional neural network, trained to identify discriminatory or representative features directly from raw pixels. Because the ground truth phenotype is unknown beforehand, representation learning is usually either weakly supervised or unsupervised. The model can be trained on a library of cellular images corresponding to different perturbations or treatment conditions and then used for feature extraction [30,51,52]. Moreover, studies have also successfully used features extracted from models trained on natural images using transfer learning [48,53]. Another approach for self-supervised representation learning is to train a convolutional neural network—based encoder to predict one of the channels in the image [54] or all channels through an autoencoder [55].

Taken together, these techniques played a major role in furthering high-throughput image-based profiling to assess and explore the effects of drug treatments [51,56], RNA interference [57—59], or other pathogenic perturbations [60,61]. More recently, researchers have also used image-based profiling to investigate the functional effects of various chemical compounds in fighting against coronavirus disease 2019 [51,62]. In contrast to visual phenotype recognition using supervised learning (Recognizing phenotypic traits of cells), image-based profiling allows analysis of visual phenotypes in an unbiased way to formulate hypotheses and understand biological models and perturbations. This approach is widely used in drug discovery and functional genomics studies and is increasingly being adopted in other applications wherein images may reveal novel phenotypes that require quantification.

Predicting visual phenotypes

Images revealing high-quality visual phenotypes can now also be generated using deep learning models. Given certain experimental observations, a model can estimate how cells would look like under specific treatments or procedures. In this way, we can run simulations, make virtual experiments, or impute missing data that are difficult to collect physically.

Fluorescence labeling reveals specific cellular structures and can make visual phenotypes easier to be detected by the human eye. However, fluorescent markers also have several limitations, including costs, toxicity for living cells, or not even compatible with live imaging. An emerging trend in microscopy is the prediction of fluorescence labels from transmitted bright-field images [8,10,63], wherein deep learning models recognize features from unlabeled images to estimate where the stains would activate inside cells if physically applied. This approach has been successfully used to screen compounds for treating Alzheimer disease, improving hit rates in a prospective evaluation [64]. It was also shown to improve throughput in high-content screening applications using reflectance microscopy, which results in more accurate virtual stain predictions [65].

Label-free images may contain more information than what can be recognized by the eye, expanding to other potential applications. The segmentation of the nucleus without a DNA stain is an example that follows a similar approach. The fluorescent marker is first applied on training images only, and then, trained models can detect the nucleus directly on bright-field images [66]. The use of imaging flow cytometry for diagnosing leukemia usually relies on several fluorescent markers, which could be used to train a model that detects the same phenotype using bright-field and dark-field images only [31].

Deep learning models can also transform low-resolution visual phenotypes into high-resolution images [67]. The frame-rate limits in super-resolution for single-molecule localization have been overcome without compromising accuracy using deep learning models that accelerate image reconstruction [68]. Similarly, super-resolution from different microscopy modalities, such as transforming confocal microscopy images to match the resolution of a stimulated emission depletion microscope, has been shown to be accurate [69]. There are still challenges with using label-free images and reconstructing phenotypes computationally, and more research is still needed to determine the reliability of these approaches beyond proof-of-concept experiments [70].

Future directions

Deep learning is expanding our ability to study cell biology with images in many different directions. In addition to recognizing phenotypic traits, profiling cell state, and generating new images, machine learning can also power cell sorting in real time [71,72], guide optimal experimental design [73,74], report uncertainty under the presence of novel phenotypes [75], and enable real-time volumetric reconstructions of cells [76]. Taken together, these applications exemplify how visual phenotypes can support and automate experimental decisions even before performing downstream biological interpretation.

Imaging is a rich source of information that can be combined and connected with other existing biological data, most notably high-resolution genetic data. Emerging technologies for sequencing single cells in tissues [77—79] offer the unique opportunity to study the relationship between visual phenotypes, gene or protein expression levels, and their spatial distribution (Box 2). Deep

learning has been recently used to predict local gene expression patterns from tissue images [80,81] and bulk mRNA levels from whole-slide images [82], all based on hematoxylin and eosin—stained histology samples. These results indicate that the correlation patterns between morphology and gene expression can be extracted and quantified, likely improving accuracy as more spatial transcriptomics data are acquired.

Another promising tool for multimodal single-cell analysis is highly multiplexed imaging, which involves measuring multiple fluorescent markers simultaneously [83]. Unlike hematoxylin and eosin staining, these techniques result in a comprehensive map of cellular organization within a tissue at single-cell resolution. Highly multiplexed imaging has already been successfully applied to obtain spatially resolved, single-cell level measurements of transcriptional and protein landscape within a tissue [84]. The computational pipeline for analyzing multiplexed imaging of tissues typically involves similar steps as in image-based profiling (Profiling cell state) [85]. The obtained features can then be used to classify cell types, analyze cellular neighborhoods, and study cell—cell interactions across phenotypes within a tissue microenvironment [86]. Although still in early phases, deep learning tools may be used for integrating cell morphology with changes in gene and protein expression to study tumor microenvironments.

One of the main drawbacks of deep learning models is that they encode visual phenotypes in multiple layers of feature maps that may not have any direct interpretation or biological meaning. Developing methods that can explain the patterns found in the data can facilitate image analysis in supervised learning and profiling applications. The use of generative models has been explored for explaining differences in phenotypes based on the underlying data distribution [55,87]. Additional work remains to be carried out in areas such as designing deep learning architectures that are inherently interpretable in a biological context [88], finding positive and negative examples associated with a phenotype of interest, and using causal inference to identify the biological effects of interventions.

Developing and training deep learning models takes significant time and resources, including data collection and computing power. The impact of visual phenotyping based on deep learning can be amplified by sharing data, code, and trained models for other laboratories to reproduce and apply on their own research. Open data and code will help advance methods, while model sharing will facilitate adoption of robust and generalist solutions. The biological imaging community is making coordinated efforts to bring these technologies to all biological laboratories for supporting their research and for making the power of visual phenotypes more accessible for biological discovery.

Declaration of competing interest

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

Acknowledgements

The authors would like to thank Anne Carpenter, Sami Farhi, Wolfgang Pernice, and Shantanu Singh for valuable discussions and recommendations made to complete this manuscript. This work was supported by the Broad Institute Schmidt Fellowship program.

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