

Artificial intelligence— assisted organ-on-chip systems

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1. Introduction

In the past few years, diverse areas in science and technology, including tissue and material engineering, biosensors, and microfluidics, have been exploited to construct more sophisticated organ-on-a-chip (OOC) devices. Such an interdisciplinary collaboration aims to reproduce in vitro physiological conditions precisely for developing advanced therapies and accelerate the translational research paradigm, where new scientific discoveries are applied in clinical practice.

Current OOC devices have swiftly extended their level of complexity and achieved a resemblance to real organs. The responses acquired on OOC devices, for example, metabolic alterations in multicellular systems upon drug treatment, are continuously monitored and recorded. Next-generation OOC devices are designed to have a high throughput where engineered or natural miniature tissues are exposed to a three-dimensional microfluidic regime. The generated data multiply with the parallelization of such devices. With the integration of sensing tools, such devices can instantly gather vast amounts of data (De Chiara et al., 2021). For example, a simple time-lapse study lasting 24 h on a confocal microscope to capture two images of microtissues or organoids (bright-field and fluorescent images) can generate hundreds of gigabytes of data. Regrettably, the current situation is such that the data produced by these platforms are beyond human's ability to process and evaluate efficiently. Consequently, owing to the immense quantity of data that needs to be processed, it has become essential to rely on computers, particularly artificial intelligence (AI) (Sarker, 2021).

Briefly, AI has revolutionized a wide range of technological fields by enabling machines to execute specific tasks that commonly demand human intelligence (Ertel, 2011). Such a tool can learn from data, adapt itself to new inputs, and perform tasks accordingly (Goertzel, 2007). AI possesses several key components, including supervised and unsupervised machine learning (ML), reinforcement learning,

computer vision (CV), robotics, cognitive computing, natural language processing (NLP), neural networks (NN), and deep learning (DL) (Crawford, 2021). There are several challenges associated with developing OOC devices, including replicating the complexity of the human body, reproducibility, durability, and overall cost that can limit their widespread adoption, scaling up, and standardization of protocols. The use of such components has garnered notable attention in OOC devices to eliminate these challenges and improve the functionality and outcome of these tools (Fetah et al., 2019). For instance, AI can continuously monitor the behavior of cells or tissues in real time and learn from a large set of drug screening data to predict if a drug is being effective or causing adverse effects on organoids (Chan et al., 2019). ML algorithms can be trained to analyze the data generated by sensors and imaging tools, providing researchers with insights into molecular processes that underlie organ functions (Mencattini et al., 2019). By monitoring cellular behavior, ML can identify specific patterns and correlations and then predict how cells will behave under different conditions. By integrating AI into the closed-loop control systems, it can dynamically adjust the parameters of the OOC device to maintain optimal conditions. AI can also be trained to monitor the effect of microfluidic conditions such as flow rates, pressure gradients, or temperature on organoids to predict the optimal conditions (Liu et al., 2021). AI is a swiftly advancing area that can revolutionize various facets of OOC technologies. AI has the potential to enhance the capabilities of OOC devices, making them more reliable for investigating human physiological conditions and diseases.

2. Adaptation of AI to OOC devices: Design parameters

The amount, size, and data type must be considered when selecting the relevant algorithm for the OOC application (Greener et al., 2022). As the size of each data element (e.g., a microscope image of cells taken from an organoid culture) inputted into the system increases, more complex and resource-intensive algorithms must be implemented (Greener et al., 2022). A rule of thumb in most ML applications is that the more data available, the better the system performs. This is especially paramount for applications where the system has to process a large volume of inputs in each iteration. However, larger datasets come with the trade-off of requiring increased computational power to process the data. Solving the issue of processing larger datasets for improved performance requires an increase in computational power (the number of CPUs, GPUs, servers, or custom-built computational elements) to the system, which can be energy-intensive and cost more or increase the processing time, which can delay the biological experiments. Depending on the nature of the experiment, different kinds of measurements (e.g., microscope images, sensory data, and gene expression data) have to be periodically harvested and coalesced into the dataset. This procedure can be both time- and resource-intensive.

To improve the performance of an AI system, it is beneficial to preprocess the data beforehand. Preprocessing can extract various features from the data, reducing

its size and enabling the algorithms to find patterns within the data more quickly (Raschka, 2015). In most cases, as readily available datasets do not exist, they must be created to implement the AI. Preprocessing can be implemented as the dataset is formed or included in the algorithm before the learning stage. The following computer vision example illustrates how preprocessing and algorithm selection go hand-in-hand for an AI application that detects organoids from colored images.

2.1 A computer vision example

Regarding the use of computer vision to identify organoids cultivated in wells of microscope images, how the size of these images impacts the necessary algorithm and training data should be explored. Digital images consist of finite sets of picture elements known as pixels. In a black-and-white digital image, each pixel (represented by an 8-bit number, computationally referred to as a byte) has a value between 0 (black) and 255 (white), correlating to a grayscale color. A 128×128 pixel grayscale square image would consist of 16,384 bytes (16.384 Kilobytes) in total. A dataset consisting of 1000 such images would require 16,384,000 bytes (16.384 Megabytes) of storage capacity. A 128×128 pixel image may not have the necessary resolution to purvey the necessary amount of information. Higher-resolution images will have more information available but will also increase the needed storage capacities. A 256×256 image will require 65.536 Kilobytes, while an 8-bit megapixel image (1000×1000 pixels) will require 1 Megabyte of storage capacity.

Colored images require even more data as each colored pixel is represented by three different numbers corresponding to red, green, and blue (RGB) color tones. An RGB image will require three times as much storage capacity as its grayscale counterpart. Larger images contain more information, enabling the algorithms, at the cost of being more complex, to detect more patterns with which they can accomplish their tasks. Artificial neural networks (ANNs) and convolutional neural networks (CNNs) are common computer vision algorithms that use neurons arranged in layers. More neurons allow for more pattern recognition, but larger images require more neurons and layers for successful outcomes. In a neural network, each neuron's output (except for the final layer) is connected to the next layer's input with modulated weights, which are adjusted during the training phase for the desired output. Weights are represented by matrices and adjusted through various matrix operations. Larger networks require bigger weight matrices and more computational operations.

The network size depends on the input size, with larger inputs requiring more neurons and larger datasets. The dataset should be at least 10 times larger than the network size. A network with three neurons can be trained with 30 elements, while a network of 1000 neurons requires a dataset of 10,000 samples. If colored images are included, the network and dataset should be three times larger.

One-megapixel colored images contain a huge amount of information with many potential patterns for the network to recognize. Let us assume that a network consisting of a total number of 3000 neurons is sufficient to detect the presence of organoids in each image. For the ANN to function correctly, it must be trained on a dataset of

30,000 images, which requires a storage space of 90 Gigabytes. Converting the images to grayscale, at the cost of losing the patterns the network will learn from color, can reduce the network size to 1000 neurons, requiring a smaller dataset (10,000 images meaning only 10 Gigabytes of storage will be required). Processing such big datasets is difficult and time-consuming for a single computer, hence requiring the integration of expensive hardware infrastructure. Generating datasets this big is also a challenge on its own.

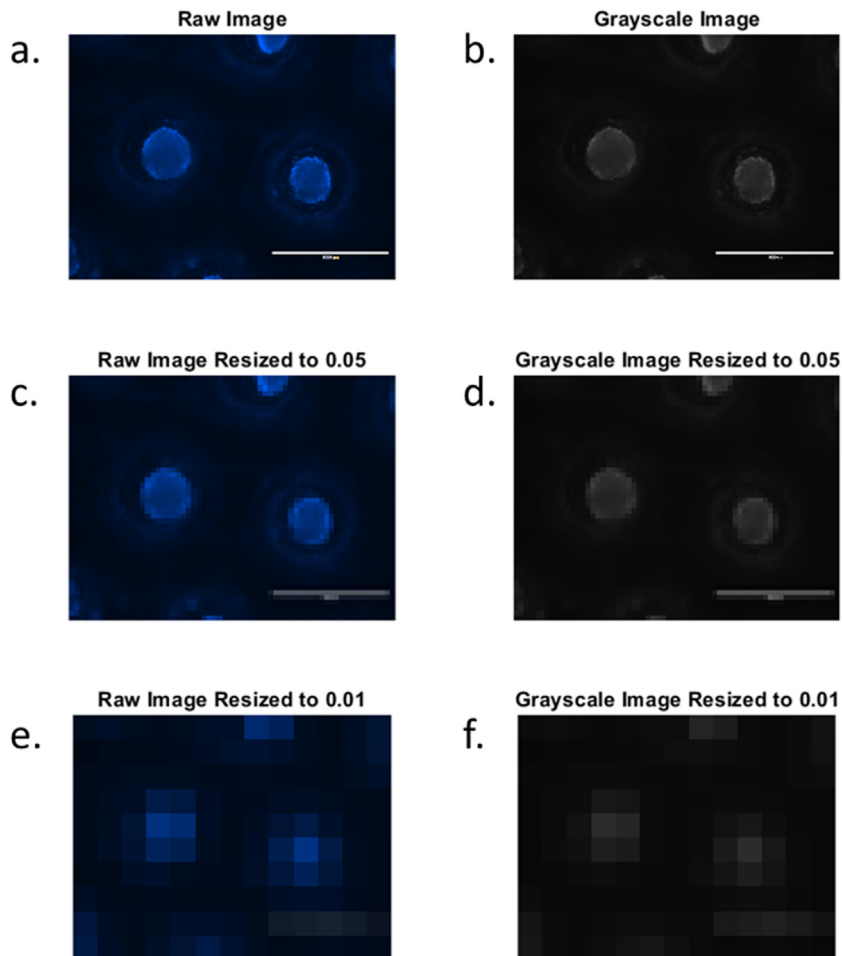
Reducing the size of the processed grayscale images down to 256×256 pixels would reduce the needed size of the network to around 100 neurons, reducing the minimum required size of the dataset to 1000 images. The smaller sizes of the images would also require less storage (around 65 Megabytes), which makes it possible to train the network on a standard computer without expensive hardware. Using smaller-sized or grayscale images does not require custom digital cameras. Using software such as MATLAB or programming languages such as Python can be used to modify the images to be grayscale or smaller. [Fig. 5.1](#) illustrates how modifying the image can alter the amount of information it carries.

3. Paradigm shift with AI-integrated organs-on-chip models

Organoids and related studies offer an accurate examination of cell environment and behavior. Manual analyses may be inaccurate and time-consuming, reducing confidence in these methods. AI provides fast and accurate analysis through deep learning, optimizing organoid growth and conducting drug studies. Image processing and deep learning are used to maintain cell distribution, classify cells, apply drugs, and examine environmental interactions.

Preserving the desired cell's homogeneity in the culture medium is one of the most critical issues in cell studies and related applications. Recognizing the structure of the targeted cell and defining and classifying it in real time on the system are one of the most powerful solutions to this parameter. The chip, which integrates deep neural networks (DNNs) and enables the identification and classification of single cells and 3D cell cultures, was developed by Anagnostidis et al. to solve this problem and perform high-accuracy cell classification ([Anagnostidis et al., 2020](#)). This platform, which learns the characteristic features of cell types and classifies them according to these characteristics, can also store the isolated cells for further analysis. Polyacrylamide hydrogel beads (PA), MCF-7, and polystyrene spheres were performed to assess the platform's detection and classification capacity of deep learning. The accuracy was over 90% for the mammalian cell identification under 10 min.

One of the common problems in 3D cell and tumor studies is the inability to evaluate the process with high accuracy. It is of great importance to assess the images with higher accuracy with the help of AI. Chen et al. were able to identify tumor spheroids, including breast cancer cells (MDA-MB-231 and MCF-7), lung cancer cells (A549 and NCI-H23), and colon cancer cells (HT29 — HCT116), and their

**FIGURE 5.1**

(A) The raw image of HEK organoids expressing a fluorescent protein taken under a microscope. The image has a 960×1280 pixel resolution. (B) The image is converted to grayscale. Information that can be gleaned from color has disappeared, but the organoids are still visible. (C) The image is reduced to 48×64 pixels (5% the size of the original image). The shapes of the organoids are still discernible. (D) Image C converted to grayscale. (E) The image is reduced to 10×13 pixels (1% the size of the original image). The shapes of the organoids are no longer discernible. (F) Image D converted to grayscale.

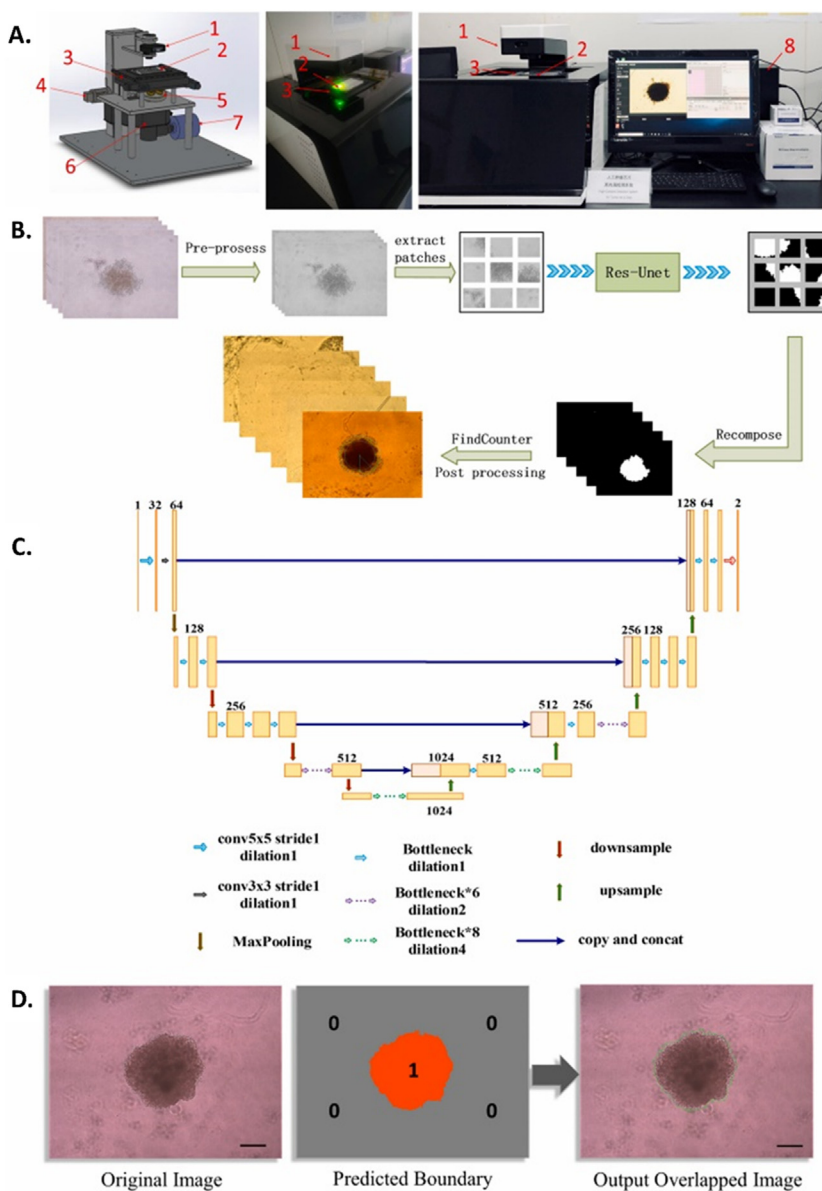
invasiveness with a neural network—based algorithm that was created by combining the excess perimeter index (EPI) and multiscale entropy index (MSEI) indices ([Chen et al., 2021](#)). These cancer cells were induced using drugs such as doxorubicin,

docetaxel, paclitaxel, cyclophosphamide, and 5-Fu. Measurement difficulties created by spheroids and extracellular matrix (ECM) components in cell culture under normal conditions are avoided by using fluorescence labeling, which requires extra processing. However, in this study, 3D analyses were performed using bright-field imaging. As a result, for example, the decrease in viability observed in lung cancers decreased to approximately 10% within 10 days with the induction of 5-Fu.

Trossbach et al. developed a deep learning—integrated platform to enhance spheroid production and characterization yield (Trossbach et al., 2022). For this purpose, a CNN was utilized to analyze, evaluate, and classify the three cell lines, including BJ-hTERT cells, 5637 cells, and RT4 cells, by characterizing their surfactant concentrations and needed incubation times for their optimum culture periods. In this process, deep learning was trained by implementing image processing. The spatial distribution of spheroids formed by the droplet cell culture method was mapped and analyzed. Based on these results, for example, it was estimated that no more than 0.75% w/v surfactant is needed for BJ hTERT cells to form spheroids. With this study performed with bright-field imaging, the required amount of substance and the developmental processes of spheroids could be analyzed.

In many studies involving deep learning, the image processing part is used to analyze the development of spheroids or their interaction with drugs. Particles can be manipulated according to their size with acoustofluidics, one of the separation methods frequently used in microfluidic systems. By combining the learning ability of AI with this particle manipulation strategy, Cai et al. achieved growing spheroids on a chip and automatically performed the necessary manipulations in this process (Cai et al., 2021). For that purpose, a mini bioreactor, which includes rotors, a camera, and a reinforcement learning-based controller, was fabricated to control the rotational speeds of organoids during the culture process and maintain the homogeneity of organoids (Fig. 5.2). On the other hand, prodigious development in neural differentiation was provided. This study optimized an organoid growing process, eliminating error-prone concepts such as manual operation.

In addition to image processing studies, AI assistance can be adopted in drug screening studies. Researchers developed a biomimetic on-chip platform that generates a highly well-represented bone model (Paek et al., 2023). The designed OOC model includes all the critical aspects of the bone microenvironment, including osteocytes, osteoblasts, and a biologically derived ECM reformed inside a 3D well. This model also aimed to be combined with antiosteoporosis drug screening studies by AI by providing a direct observation scheme for bone maturation and bone—cell differentiation. Here, a CNN generates a neural network frame to process the response of the anti-SOST antibody, which regulates the Wnt pathway and controls osteoblast growth. Response of the drug was also calculated by the integrated fluorescence imaging of 3D wells with relevant β -Catenin translocation (with Alexa Fluor 488 conjugated antibody labeling) to the cell nucleus (with Hoechst 33342 dye) as a result of the antiosteoporosis drug. The study demonstrates that employing specific strategies enables the effective training of neural networks using extensive

**FIGURE 5.2**

Analysis of the boundaries of invasive tumor spheroids by utilizing a deep learning algorithm. (A) The SMART system was improved for automated image processing and measurements. (B) Execution steps were represented. (C) The frame of PSP-U-Net was illustrated. (D) Image analysis was conducted using PSP-U-Net to predict the spheroids' boundary (Chen et al., 2021).

datasets. Consequently, this approach holds promise for establishing a reliable system to assess drugs comprehensively in large-scale screening studies reliant on image processing.

Time Lapse Microscopy (TLM) is an essential tool in biological studies to observe bioprocesses directly. Nevertheless, the ability to record high-resolution video is a drawback in most setups. Literature studies have been conducted to overcome this problem by adopting machine learning algorithms to generate superresolution recordings from any type of record obtained. In another study, Cascarano et al., 2021, generated a machine learning algorithm to achieve superresolution in TLM records without prior training (Fig. 5.2A) (Cascarano et al., 2021). By improving the well-known Deep Image Prior (DIP), a new algorithm was developed to obtain high-resolution recordings from prerecorded, low-resolution OOC data. This study's outputs can potentially improve existing diagnostic and screening systems integrating the OOC approach.

4. Conclusion

Integrating AI with OOC systems marks a pivotal leap in studying human physiology and advancing drug discovery. Through machine learning and neural networks, AI efficiently manages vast data, predicts behaviors, optimizes conditions, and accelerates drug testing. From identifying cells in real time to enhancing drug screening, AI revolutionizes OOC devices, offering accurate analyses, optimizing growth conditions, and streamlining drug studies. These advancements, exemplified by deep learning algorithms, enable precise evaluations, classification of cells, and even superresolution imaging, propelling OOC technology toward robust, efficient, and reliable applications in personalized medicine and biomedical research.

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