Automated 3D cell organoid manipulator robot for precision cancer therapies using deep learning (A3DCOMRFPCTUDL)

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ABSTRACT

3D cell cultures are predicted to be the next generation of drug discovery models since they can better model disease physiology. The drug testing benefits from a standardized environment which means that the selected 3D cell cultures should be as similar to each other as possible to get the most precise results. We propose an artificial intelligence-guided low-cost 3D cell culture delivery system. It consists of a stereomicroscope, a micromanipulator, a syringe pump, and a computer. The system automatically performs morphology-based feature analysis on spheroids and transfers the selected ones between various sample holders.

Keywords: 3D cell cultures, deep learning, automatization

1. INTRODUCTION

- In drug discovery studies, cell-based assays have been widely adopted as a model system. Two-dimensional (2D) model systems are still in use, to test compound activity.
- It has recently been described that three-dimensional (3D) cell cultures are more relevant model systems for drug testing, because they enable the examination of drug penetration and tumor development. One of the most common 3D cell cultures is the spheroid models where the cells form a sphere-like structure. Spheroid models can reflect *in vivo* conditions better.
- High throughput analysis of spheroids is still a challenge, and standardizing experiment conditions has not been solved yet.

Most of the cases pre-selecting spheroids by their morphology is a very time consuming and inconsistent process because it requires human assistance. Spheroids derived from cell lines and patients will result in different shapes and sizes that can make the selection difficult. There is a need to standardize the process of spheroid manipulation.

In this work, we propose a new solution to work with spheroids. We designed and built an automated microscope that standardizes the conditions of experiments. The device can support or fully replace experts by automatically selecting and transferring 3D cell cultures for further analysis. We developed a fast and accurate deep learning-based framework to detect and segment spheroids and integrated it into the machine's controller software. Using such accurate segmentation, the algorithm is able to extract features robustly and - based on user defined criteria - decide which spheroids to manipulate. This system was developed to standardize and automate one of the most time-consuming processes during sample preparation, the pre-selection phase, where accuracy is crucial.

2. STATE OF THE ART

Nowadays there are many different techniques available to assemble 3D cell cultures [1]. However, the lack of a unified protocol for creating 3D cell cultures, such as spheroids, results in varying shapes with huge morphological heterogeneity that can lead to false clinical studies. Moreover, imaging of spheroids is limited because of the light scattering due to their compact structure [2]. Apart from the light scattering, the type of the screening plate can also greatly affect the image quality. For example, U-shaped bottom plates are suitable for growing spheroids because they will have more beneficial, spherical morphologies, but negatively affect imaging quality. Therefore it might be required to

transfer these spheroids to a flat surface, e.g. a flat bottom plate or a Petri dish.

The imaging conditions of spheroids can strongly vary, such as light, illumination, density of the medium, or the shape of the plates that can affect the expert's decision during the pre-selection phase. Spheroids should be detected and segmented on label free brightfield microscopy images. Current methods that handle such images are based on classical analysis methods like thresholding or watershed [3]. Most recently, deep learning object detection and segmentation methods become popular since they provide more accurate results, and do not require fine-tuning even if the imaging conditions change [4]. Although deep learning-based object detection has already been used for cell detection and segmentation, there is no existing model for spheroids.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

Selecting spheroids for clinical studies is a manual process and it can be inconsistent due to human objectivity. The proposed device creates a standardized protocol for spheroid handling which is absolutely necessary for drug screening experiments.

The system consists of a stereomicroscope, custom built micromanipulator, a syringe pump, and a computer. The components are controlled from a single, custom software. The software performs four major purposes: 1) complete hardware control, 2) automated imaging, 3) spheroid detection using a trained mask R-CNN [5] deep learning framework, and 4) an easy to use graphical user interface to define custom spheroid selection and transfer criteria.

We created an annotated image database to train models for spheroid segmentation. The image processing methods used for the detection and selection are very reliable, and we show that it can outperform human selection skills. The machine can perform semi- or fully-automatic transferring of spheroids to any predefined well plate. To the best of our knowledge, there are no existing robotic systems that are designed especially for spheroid manipulation.

Size or morphology of spheroids is a very crucial property for getting accurate results in many different types of experiments. For example, in clinical trials, if the spheroids are not similar to each other in shape or size, then it is almost impossible to identify the drug penetration. Moreover, the diverse spheroid size can easily lead to the misinterpretation of the metabolic assays.

4. PROJECT RESULTS

The main parts of the hardware are a stereomicroscope, a micromanipulator, a syringe pump, and a computer (Fig. 1).

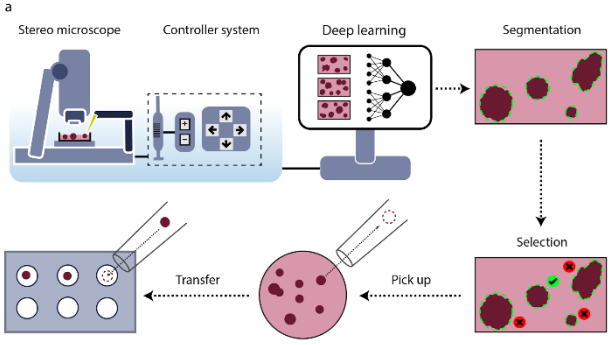

Fig. 1. Schematic representation of the SpheroidPicker. The system includes a stereomicroscope, syringe, stage, and manipulator controller. The automatic screening function takes brightfield images of the spheroids. The segmentation and feature extraction steps are based on a deep learning model. After the selection of the spheroids the spheroid picker automatically transfers them into the target plate.

The large field of view of the microscope allows the efficient and fast screening of the samples. Various well plates and Petri dishes are supported so that wells or regions can be selected or excluded from analysis. The movement area of the motorized stage is large enough to place the source and target plate next to each other, and switch between them easily. The micromanipulator moves a glass capillary rod that is used for transferring the spheroids. Its axes can be configured to move the capillary based on image coordinates. The capillary is attached to an automated water-based syringe system. Except for the microscope, the stage, and the camera, all the hardware elements are custom built. The components are controlled from a single, custom software run on a computer.

The automatic screening function results in brightfield images of the spheroids (Fig. 2a). The segmentation and feature extraction steps are based on a deep learning model and the result shown on (Fig. 2b). After the selection of the spheroids, the spheroid picker automatically transfers the spheroids into the target plate.

The transferring capabilities of the system were tested by moving them one by one from a source 96 well plate to a target plate of the same type. The selection criteria was an area range between 21,000 and 29,000 μm^2 and a minimum of 0.815 circularity. A minimum circularity criterium ensures that the selected object has a rounded shape. First, we performed this experiment such that the outcome was evaluated right after every transfer and possible issues were fixed (e.g. removed spheroids that stuck in the capillary). From 28 attempts, 26 spheroids were successfully picked up, 25 were transferred properly, and always one object was picked up, which leads to a success rate of 89%. Next, we let the system

fully automatically select and transfer spheroids between the plates. The Picker scanned a user-defined area of the sample and analyzed it. Out of 30 attempts, 24 were successful which leads to an 80% success rate.

We compared the selection capability of the Spheroid Picker to that of the manual process of our expert with the following experiment. First, we asked the expert to manually select preferably circular spheroids of about 25,000 µm² area under the microscope with a regular laboratory pipette and transfer them separately to a well of a 96-well plate. Then we set the same requirements in the software and transferred spheroids using the system in the order it offered them. Both the expert and the device transferred 42-42 spheroids. Afterwards, we used our system to determine the size and circularity properties of the manually transferred objects. The average area was 25,347.2±3,226.3 µm² in the manual case and 24,222.6±1,957.7 µm² in the automatic, while the circularity value was 0.8504±0.0147 and 0.8527±0.0150, respectively. This shows that the manual approach has 1.648 higher standard deviation in terms of area, and is less reliable when specific features are required.

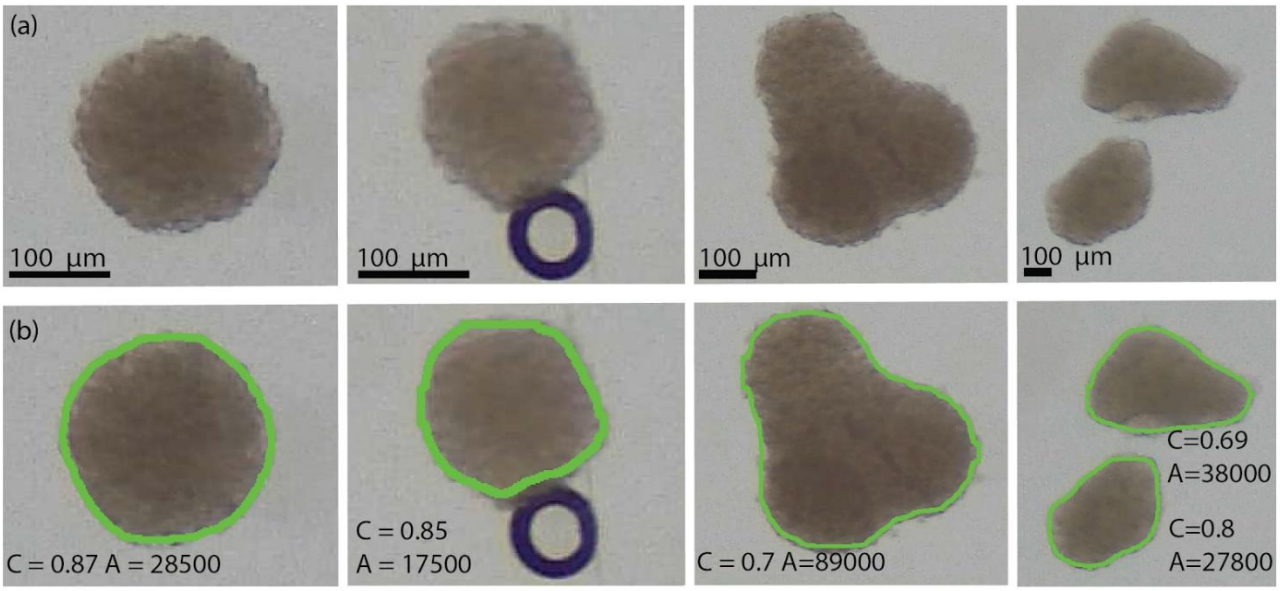

Fig. 2. Brightfield images of spheroids with various shapes and sizes (a) and their segmentation results (b). "C" value means circuralty, "A" is the area of the segmented object in µm².

5. FUTURE PROJECT VISION

5.1. Technology Scaling

Currently the prototype of the manipulator and the pipetting system has been validated in a relevant environment (TRL5). When COVID situation allows, we will ship and set up prototypes at the partner FIMM EMBL Helsinki and to Europe's most relevant research institutes to get feedback from the operational environment. The deep learning algorithm will be refined to increase robustness and efficacy (TRL6). The SpheroidPicker will be built into a custom designed microscope and will be a closed system. The software and the GUI will be upgraded to industrial level (TRL7). More new system prototypes are shipped to early customers for functional and usability feedback. Based on this information the SpheroidPicker will be finalized (TRL8).

5.2. Project Synergies and Outreach

We plan to extend our consortium with an industrial partner for the further developments and improvements of the system to make it market-ready. Many ATTRACT funded projects in the Data acquisition systems and computing, or the Sensors domain would also be good partners. Our shared aim will be to reduce the size and the cost of the base microscope to make it more compact and affordable. An industrial partner could provide useful insights in making the system user-friendly and marketing. We are currently partnered with Leica and we plan to keep working together.

Moreover, we will cooperate with further biological laboratories to discover more applications and needs. The main profile of our current partner FIMM-EMBL is personalized precision cancer drug repurposing where they use 3D cell cultures. We will introduce our system

to another collaborator in the COMPASS (ERA PerMed) consortium who is working in paediatric precision oncology. We aim to have partners for 4 different applications to whom we provide a device and collect feedback. This would allow us to optimize the device for various needs and support the society by speeding up research in cancer and personalized medicine.

5.3. Technology application and demonstration cases

We will apply the developed device in multiple scientific research projects related to healthcare. The FIMM-EMBL partner of the project specializes in personalized precision cancer drug repurposing. We will study the drug penetration in 3D cell cultures that are derived from patients to build models of concentration effects. Drug screening studies on 2D cell cultures give preliminary information of the required concentration. However, 3D models provide a more relevant condition to study the transportation effects and better adjust the dosage.

With another collaborator (Dr. Sina Oppermann, Hopp Children's Cancer Center Heidelberg) we will apply our system to discover unexpected drug efficacies and drug repositioning opportunities. This will help us to investigate whether the tumor tissue is responsive to a library of clinically approved drugs, adding a valuable dimension to diagnostics for children with cancer.

Furthermore, we are planning to use our system for viral entry studies. Previously, with our collaborators we have studied the connection between the genes and the viral entry mechanism and found that influenza A virus (IAV) takes advantage of the host cell's aggresome formation and disassembly machinery. In this project, we will study the viral infection rate in 3D cell cultures to identify treatments that prohibit viral spreading. Besides IAV, we plan to extend this study to Dengue and SARS-CoV-2.

5.4. Technology commercialization

We are in the process of filing an EU patent. Ideally, we would outsource further TRL developments and marketing to a partner company and we would focus on finding scientific users and collaborators. However, if the investment will not make this possible right away, we would apply to incubators and aim for venture capitals.

5.5. Envisioned risks

Identified risks:

- (low) Possible appearance of well plates which can also be used for growing and imaging. There would still be applications when spheroid handling is necessary, thus we would need to support the new plate as well.
- (medium) Another spheroid handling device appears in the market, as 3D cell cultures gain increased attention lately. We will have to ship prototypes fast and keep improving the features.
- (medium) Development of a custom microscope to make the system more compact would be too costly and time consuming. We will have to rely on capital investment to ship prototypes to early adopters.

5.6. Liaison with Student Teams and Socio-Economic Study

We have enabled 2 MSc students to actively participate in the development who later enrolled in PhD schools. We will keep being connected with universities and allow students to work on related topics and complete their internships or write theses in the future. Also, we are open to participating in socio-economic studies of the ATTRACT ecosystem and providing technology impact data acquired from our early adopters and customers.

6. ACKNOWLEDGEMENT

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7. REFERENCES

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