Automatic imaging of multiple spheroids by synchronous control of linear sample stage and dynamic OCT instrument

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Note: 3-page abstract can be found on the next page.

100-word abstract

We present an automated sample targeting system (ASTS) integrated with dynamic optical coherence tomography for high-throughput imaging of in vitro tumor spheroids in a 96-well plate. The system combines a Python-controlled stage-control subsystem and a LabVIEW-based OCT system, synchronized via TCP/IP communication. The proposed system successfully performed fully automatic targeting and imaging of 15 spheroids. However, two spheroids appeared out of focus and/or the FOV. These limitations are going to be overcome in the future by adding Z-stage control and neural network-based segmentation for fine positioning. The proposed system could become a valuable tool for high-throughput anti-cancer drug screening.

250-word abstract

Three-dimensional tumor spheroids are key cancer research models, as they accurately mimic solid tumor architecture and microenvironments. These models are particularly valuable for evaluating the effectiveness of anti-cancer drugs, which necessitates monitoring spheroid structure and viability over time. For tumor spheroid assessment, the imaging modality must be label-free, 3D, and capable of high-speed and high-throughput imaging. Dynamic OCT is widely used for label-free and 3D tumor spheroid evaluation. However, its ability for throughput imaging is limited due to manual operations (i.e., manual sample targeting and focusing).

We introduce an automated sample targeting system (ASTS) integrated with DOCT for high-throughput, label-free imaging of tumor spheroids in a 96-well plate. The ASTS comprises a Python-controlled stage-control subsystem and a LabVIEW-based OCT system, coordinated via TCP/IP communication to perform fully automated, sequential imaging of preselected wells. We validated the system's performance by imaging fifteen MCF-7 breast cancer spheroids and successfully acquired DOCT data for thirty samples without any manual control. The result exhibited characteristic dynamic signal patterns, such as lower activity in the spheroid core, consistent with the formation of necrotic regions. Two spheroids appeared out of focus and/or the field of view, which highlighted the need for fine focusing and real-time image-guided targeting.

Overall, our proposed system enables rapid, hands-free imaging of in vitro cancer models. With future extensions including automatic depth positioning of the sample using Z-axis stage, neural network-based segmentation, and fast DOCT protocols, this system could significantly accelerate drug response studies and facilitate high-throughput anti-cancer drug screening.

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1. INTRODUCTION

Tumor spheroid is a three-dimensional(3D) culture of cancer cells that closely emulates the structure and microenvironment of in *vivo* solid tumors [1]. Therefore, tumor spheroids have been widely used for anti-cancer drug investigations [1]. The efficacy of these drugs can be assessed by their impact on the spheroid's morphology and viability.

High-throughput and label-free imaging is essential for efficient and reproducible analysis of the spheroids drug response [2–4]. Dynamic optical coherence tomography (DOCT) is a label-free functional extension of OCT that has been widely used for tumor spheroid imaging and its drug response analysis [1]. Despite the DOCT is a label-free modality, its ability for high-throughput imaging is limited. This is because the manual and frequent sample targeting and repositioning require a long time. According to our experience, it takes approximately 5-minute measurement time to image a single spheroid in a 96-well plate, where the measurement time consists of the times for manual targeting of the sample and data acquisition. It leads to an 8-hour measurement time to image all samples in a 96-well plate, and it makes high-throughput imaging with a reasonable time resolution impossible.

In this study, we integrate a fully-automatically controlled 2-axis linear sample stage into a dynamic-OCT imaging system. This system is applied for the imaging of multiple spheroids in multiple wells of a 96-well plate. We successfully automatically obtained dynamic OCT images of 15 breast cancer (MCF-7) spheroids located in three different rows and five columns of a 96-well plate.

2. SYSTEM AND OPERATION

2.1 System configuration

Figure 1 shows the photograph of the automatic-sample-targeting and imaging system. The subsystem of the motorized sample stage (stage-control subsystem) consists of a motorized XY linear stage (MLS203-1, Thorlabs Inc., NJ) driven by a brushless DC servo motor controller (BBD302, Thorlabs).

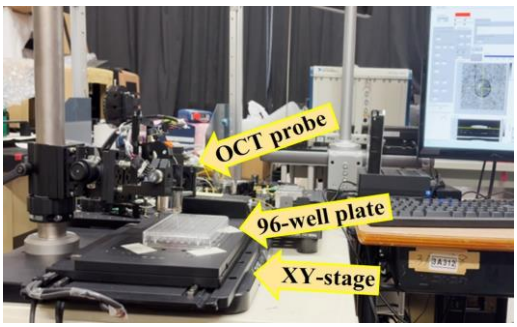

Fig 1: ASTS integrated with the DOCT microscope

The OCT subsystem is based on a MEMS-VCSEL-based 1.3-μm swept-source OCT device, operating at a speed of 100,000 A-lines/s. The objective used was LSM03 (Thorlabs), which has a focal length of 36 mm and provides an axial (in tissue) and lateral resolutions of 16.3 μm and 18.1 μm, respectively. For DOCT imaging, a lateral imaging field of 1 × 1 mm^2 is divided into 4 blocks, and each block consists of 32 B-scan locations [1]. Each block is raster scanned 32 times in 6.48 ms. Hence, at each B-scan location, 32 repeated frames are captured with an inter-frame interval of 207.36 ms. The OCT volume comprises 128 locations. For each volumetric DOCT measurement, a full three-dimensional set of interference signals is acquired in 26.4 seconds and stored in a hard disk (HD) storage. The interference signals are later processed to reconstruct the DOCT volume.

Each sample is located in each well of a 96-well plate (schematically shown in Fig. 2), where 96 wells are aligned with 9-mm spacing (from the well center to the well center). The traveling path over multiple wells (exemplified by the red line in Fig. 2) is pre-set by the operator as a list of indexes of wells to be visited, and wells are visited one by one during the automatic measurement process.

The stage-control subsystem is controlled by a custom Python program, while the OCT subsystem is controlled by LabVIEW. These systems communicate with the TCP/IP protocol. During the automatic measurement sequence, the stage-control subsystem first controls the XY stage to target the first well and then sends the trigger signal to the OCT subsystem via the TCP/IP channel. Once the OCT subsystem receives the trigger, it acquires a volumetric data set and streams it to the HD. The stage-control subsystem keeps watching the data-file size on the HD until it reaches the target file size. Once the acquisition of one well is completed, the stage-control subsystem moves the stage to the next well and sends the trigger again to the OCT subsystem. Namely, no human intervention is required for the full measurement process.

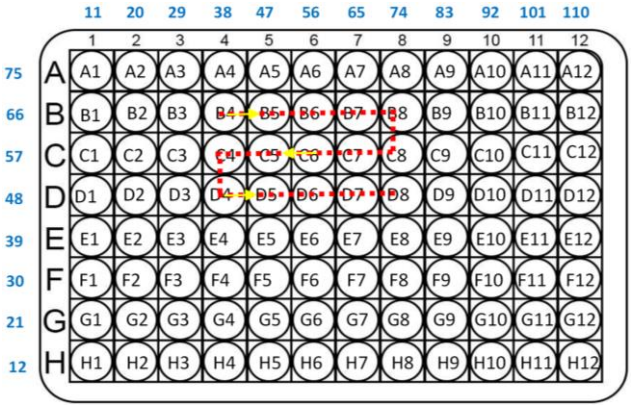

Fig 2: Schematic of a standard 96-well plate with position coordinates and highlighted the raster well-visiting pattern followed by the XY-stage

2.2 Sample preparation and scanning protocol

The operation of ASTS is validated by imaging 15 MCF-7 spheroid samples of approximately 500 µm size. 1,000 human breast cancer cells (MCF-7 cell line) were seeded in 15 wells (as highlighted in Fig. 2) of a U-shaped bottom ultra-low attachment 96-well plate to form spheroids. After 6 days of cultivation, the 96-well plate was extracted from the cultivation environment for the automatic OCT measurement.

In the present demonstration, we put 15 spheroids in the 15 wells in the well plate. During the automatic measurement, these 15 wells are sequentially visited with the path shown in Fig. 2 (red dashed line).

2.3 DOCT algorithms

To assess the intracellular dynamics, we used two DOCT algorithms: logarithmic-intensity variance (LIV) and OCT correlation decay speed ($OCDS_l$) [1]. The LIV is computed as the time variance of the logarithmic (dB-scaled) OCT intensity. And it is sensitive to the occupancy of the dynamic scatterers within the tissue [5]. The $OCDS_l$ is defined as the slope of the autocorrelation decay curve of the sequentially captured OCT frames at each B-scan location at a delay range of 512 pixels. $OCDS_l$ was supposed to be sensitive to a certain velocity range of the intracellular scatterers [5].

The raw interference signals stored in HD during the automatic measurement process were processed after the measurement session to reconstruct the LIV and $OCDS_l$ volumes.

3. RESULTS

Figure 3 shows the fully automatically acquired OCT and DOCT images of the 15 measured spheroids. The bright field micrographs (top row) were acquired before the automatic measurement session for a reference. Out of the 15 spheroids,

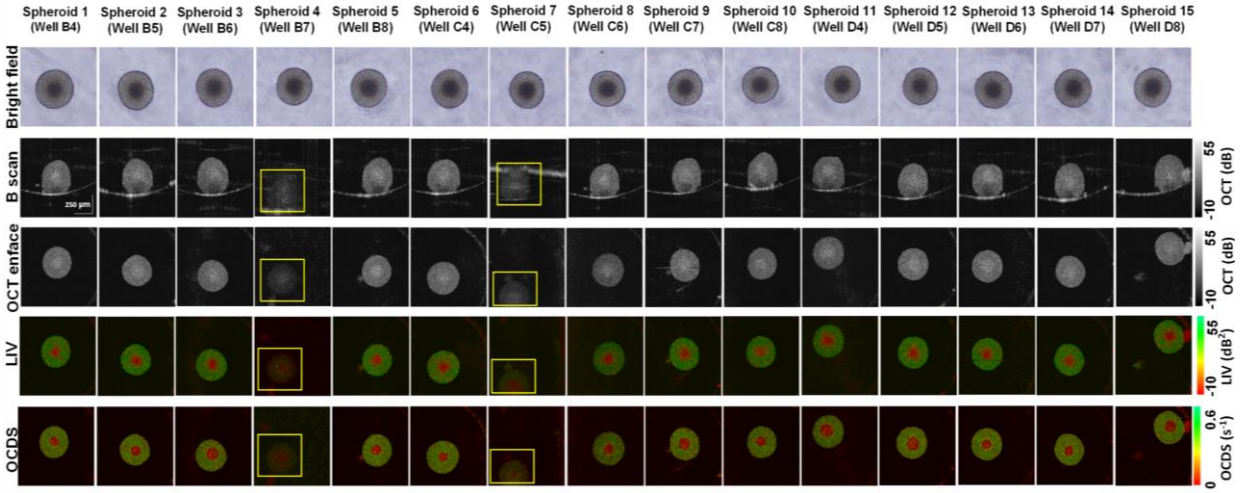

Fig 3: Automatically captured OCT and DOCT images of MCF-7 spheroid. The panel includes bright field, OCT B-scan, and enface, and the corresponding LIV and OCDS enface images of 15 MCF-7 spheroids acquired automatically. The spheroids that were out of focus or out of the field of view (FOV) are highlighted by the yellow squares.

13 were successfully imaged using the automated system. And they exhibited the well-known DOCT patterns of the spheroid. Namely, the spheroid center exhibited reduced LIV and $OCDS_l$ signals (red) [Fig.3 (4^{th} and 5^{th} rows, respectively)], which collocated with the dark appearance in the bright field images [Fig.3 (1^{st} row)]. It may indicate the well-known necrotic core of the spheroid, likely caused by hypoxia and limited nutrient availability [1].

The remaining two spheroids (highlighted by the yellow boxes in Fig.2) do not show a clear contrast in OCT. It might be because they were out of focus and/or out of the field of view. The out-of-focus issue can be attributed to the variations in the culture medium volume among the wells. On the other hand, the out of the field of view issue might be attributed to the slight shift in the spheroid location. Namely, the spheroid was not exactly located at the centre of the well. To overcome these limitations, further extensions of the proposed ASTS system are required, as discussed in the Discussion section.

4. DISCUSSION

4.1 Further extensions of the current system

As discussed in the result section, out of the 15 spheroids imaged, two spheroids were out of focus and/or out of the field of view. Those spheroids were likely in different depths and were relocated from the centre position of the well. The intra-well variations of the culture medium amount lead to a slight change in the focus position among the wells. To address this limitation, two extensions of the system are being considered. One is the integration of an axial translation stage, which will enable the relocation of the sample into the correct depth. The other is robust segmentation of the spheroid, which enables image-based feedback and precise targeting of the sample. Our colleague, Yuping Zheng, will give a poster presentation in the BiOS 2026 OCT conference about the neural-network-based robust segmentation of spheroids.

The ultimate goal of this system is to enable automatic time-lapse imaging of a large number of spheroid samples. For this purpose, the current data acquisition time (around a minute per spheroid) is not short enough. We are going to apply an NN-based high-speed DOCT imaging method, which requires only 4 repeated frames [6]. It will enable a very short volumetric DOCT acquisition time as short as 6.5 s/spheroid. The combination of this high-speed DOCT method and the automatic sample targeting method will enable the all-sample imaging of a 96-well plate in as short as approximately 15 minutes.

5. CONCLUSION

We demonstrated an automated multi-sample imaging system which integrates the sample-stage-control subsystem and the OCT subsystem. These subsystems communicate via a TCP/IP channel and enable automatic measurement without manual intervention. A set of 15 spheroids in a 96-well plate was successfully imaged. Among them, only two spheroids appeared to be out of focus and/or field-of-view. These limitations are going to be addressed by integrating an additional depth-axis (Z-axis) stage to tune the depth position of the sample and establishing an image-based segmentation method, which will enable image-feedback-based 3D positioning of the sample. The proposed system might be a useful tool for high-throughput spheroid-based anti-cancer drug screening.

6. REFERENCES

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