Novel Optical Coherence Tomography (OCT) technology for non-invasive 3D imaging

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Introduction

Background:

3D *ex vivo* platforms are being diligently evaluated as better predictive drug efficacy testing tools in preclinical as well as clinical space in the quest for profiling of novel anticancer entities (as single or two drug combinations). Within this context, there has been growing need in improved imaging and analysis of complex 3D structures. Optical Coherent tomography (OCT) has been widely used as one of the most important test in ophthalmology. It is a non-invasive imaging technology that renders the high resolution and cross sectional images from retina. Given its tremendous use in in vivo application, in recently, the technology has been applied in 3D *in vitro / ex vivo* applications for performing imaging of spheroids/ organoids and large tissues. This technology allows to perform large tissue imaging, non-invasive monitoring of macro and sprouted neo-vasculature without the need for fluorescent staining for providing quantitative information about the vascular morphological changes, thereby allowing for the evaluation of anti-angiogenic drugs in real time.

Aim:

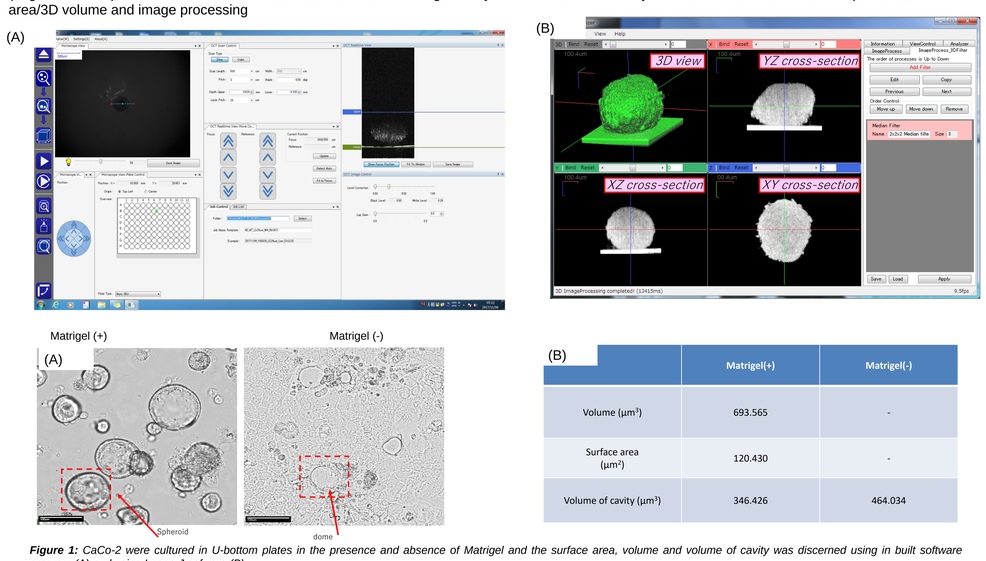
To develop a novel OCT based technology for imaging and analysis of 3D structures, such as, spheroid /organoids, tissues for growth & morphological evaluation, quantification of internal cavities, drug sensitivity testing to capture the events leading to tumor cell death and assessing effect of anti angiogenic drugs.

Material & Methods and Results:

All cells were cultured in a suitable manner, and cells were imaged by spectrum-domain OCT (SD-OCT) system. The SD-OCT system is outlined below. The samples were imaged from the bottom surface. Original images obtained by the OCT system contained noise (such as from the collagen gel surrounding the sample). To reduce this noise, image processing was applied for the collected original OCT images using original our software (SCREEN) and ImageJ image processing software (NIH) as follows. The images were subsequently processed with filters. The images were then converted into binary images so that the cell area is white and all other areas are black. Each feature values (volume, cavities etc…) were analyzed.

Optical Coherent Tomography (OCT) work flow and image acquisition

Panel A: Depicts the interface window where the settings including selection of plate format, threshold for the upper and lower limit of the sample (Organoid/tissue) is delineated. Panel B: Illustrates the sectional image of any direction: 3D view of any direction: distance between two points: 2D sectional area/3D volume and image processing



(B)	Matrigel(+)	Matrigel(-)
Volume (μm³)	693.565	-
Surface area (μm²)	120.430	-
Volume of cavity (μm³)	346.426	464.034

Figure 1: *CaCo-2 were cultured in U-bottom plates in the presence and absence of Matrigel and the surface area, volume and volume of cavity was discerned using in built software program (A) and using Image J sofware (B)*

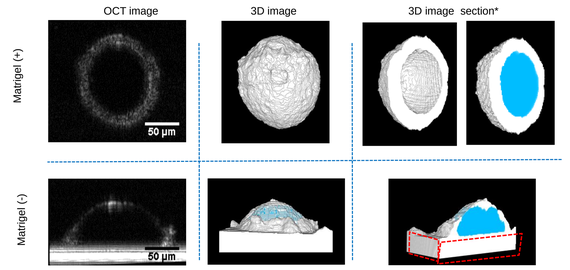


Figure 3: CaCo-2 were cultured in U-bottom plates in the presence and absence of Matrigel and the surface area, volume and volume of cavity was discerned using in built software program and Image J software.
*__Blue__ indicates cavity __Red__ indicates the bottom of plate

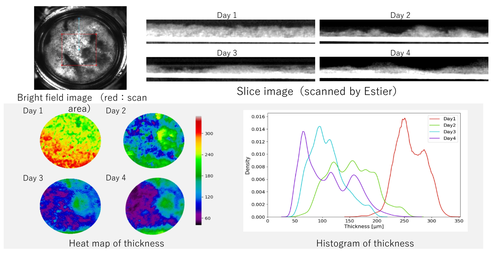
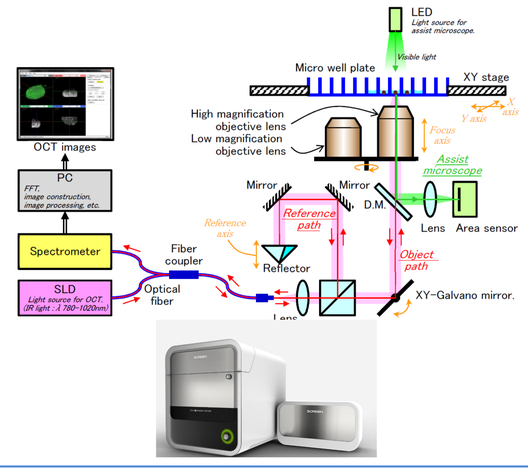


Figure 4: Normal Fibroblast cells, NHDF were maintained in the cell culture insert, and cells were stacked to male cell sheet. The thickness of sheet was quantitated in a label-free manner using Python. Heat map and histogram indicate thickness of sheet.

OCT Optical System

SCREEN HOLDINGS has developed novel Optical Coherent Technology (OCT) for morphological evaluation of complex 3D structures and tissues. The system is equipped with an 850-nm light from super luminescent diode (SLD). The OCT observation system adopts an inverted microscope, which picks up the reflected light from sample. (mirror reflected or backscattered). It is similar to reflection microscope or confocal microscope.



Efficacy Testing in Microtumors Derived from HEK293T cell line

HEK293T cells were prepared and seeded in 96 well plates. The cells were treated with Chetomin and incubated at 37ºC for upto 24-48 hr. The morphological evaluation of the spheroids derived from HEK293T cells treated and untreated was performed in a Time-lapse measurement using OCT. Panel (A) shows the spheroids cultured in the absence of Chetomin and Panel (B) represents the Chetomin-treated spheroids.

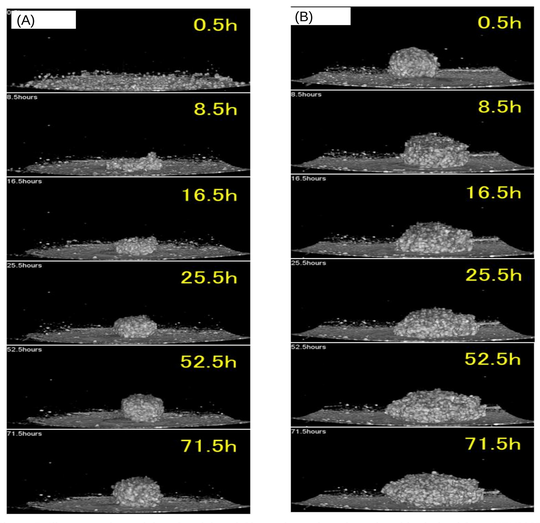


Figure 5: Illustrates the activity of activity of Chetomin against the spheroids derived from HEK293T cells: *The time course measurement of activity of chetomin shows that the treated spheroid gradually loose its circularity in comparison to the untreated spheroids.*

3D OCT imaging vs. Light sheet microscopy

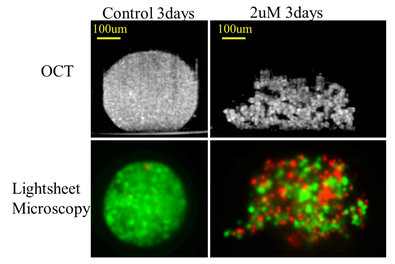


Figure 6: MCF-7 cells tagged with EGFP were cultured in U-bottom plates and treated with 2 μM of A23187 (ca ionophore). The cells were further incubated for 3 days until the cells were organized into spheroids. Subsequently, the spheroids were imaged by OCT as well as Light sheet microscopy to delineate the drug efficacy , shown as loss of circularity.

Extracted from: Tamio Mizukami et al. (Nagahama Institute of Bio-Science and Technology, *2: Frontier Pharma,)

Assessment of Angiogenic sprouting with OCT

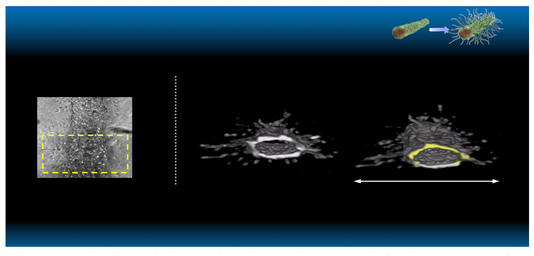
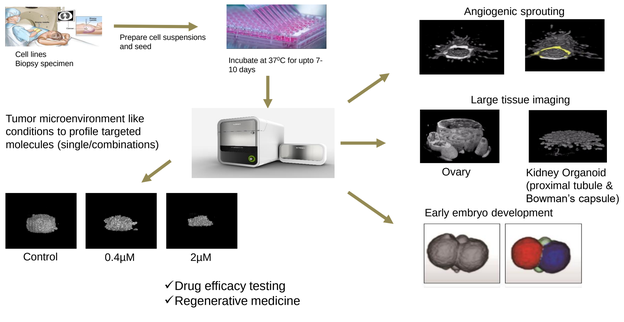


Figure 7: Depicts the analysis of angiogenic sprouting using optical coherence tomography (OCT). Yukiko T. Matsunaga et. al., (Cent. for Int'1 Res. on Integrative Bio. Med Sys., Insti. of Ind. Sci., Univ of Tokyo, Japan)

Applications and workflow



✓Drug efficacy testing
✓Regenerative medicine

Conclusions

- ❖ Non-invasive IR laser technology based Cell 3imager, ESTIER can be used for True 3D imaging of complex 3D structures, using Spheroids/Organoids and large tissues.
- ❖ The time lapse measurement delineates the activity of the drugs and effects on the morphological aspects in spheroids when treated with Chetomin and A23187.
- ❖ The technology can be utilized in quantitation of size, volume and internal cavities of various complex 3D structures.
- ❖ High utility for multiple applications in Oncology, regenerative medicine.
- ❖ User friendly work flow for analysis of data with automation capability.
- ❖ Complementary system for exiting microscopic and high content imaging systems.

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