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ENGINEERING FOR HEALTH

Label-free Volumetric Imaging of Synthetic Cell Chassis using Optical Coherence Tomography

Bottom-up, chemically formed synthetic cells are usually imaged by optical microscopy, and the cell sizes and shapes are mostly estimated from acquired 2D images. The three-dimensional (3D) structures of a compartmentalised synthetic cell can be analysed by axially stacking 2D images, typically by using a high-resolution imaging systems, such as laser confocal scanning microscopy and light sheet microscopy. However, these techniques require the synthetic cell to be labelled with fluorescent tags, and have performance limits such as being restricted to volumes less than (approximately) 200 μm^3. Here, we present the label-free, 3D imaging of soft, free-standing, multicompartment synthetic cell using optical coherence tomography (OCT). The volumes of sub-cellular compartments within individual synthetic cells can be obtained via OCT imaging measurement. The spatial arrangements of the compartments and their contact angle information can be illustrated and measured, respectively. This approach provides a new method to evaluate multiphase soft materials spanning the range of micrometres to millimetres, towards the optimisation of synthetic cell construction for novel biomimetic material development.

Keywords:
Optical coherence tomography, synthetic cells, soft materials, volumetric imaging, contact angle measurement.

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P. Dimitriou, O. Castell, J. Birchall, and J. Li, 'Label-free Volumetric Imaging of Synthetic Cell Chassis using Optical Coherence Tomography', *Proceedings of the Cardiff University Engineering Research Conference*, Cardiff, UK, 2023, pp. 15-18.

doi.org/10.18573/conf1.e

INTRODUCTION

One goal of synthetic biology is to apply engineering principles to devise new biological devices and artificial biological systems [1]. Bottom-up synthetic cells can be formed from water-in-oil emulsion templates and fabricated as soft microcapsules using microfluidic approaches [2,3]. Such constructs can encapsulate cellular metabolic reactions and biological processes, to mimic natural cell functionalities, controlled with well-organised, fluidic, cellular compartments [4–6]. This paves the way to develop next-generation biotechnology tools and novel biomimetic materials, for new pharmaceutical and healthcare applications [7,8].

The imaging of synthetic cells and other droplet-based materials are typically undertaken using a fluorescent imaging system, such as laser confocal scanning microscopy, total internal reflection fluorescence microscopy and light sheet microscopy [9,10]. These are widely applied in biophysics studies to determine artificial membrane properties, identify intercellular communications through reconstituted protein channels, and monitor the process of cell-free synthesis in droplet networks. Optical coherence tomography (OCT) provides a non-invasive, label free, and rapid imaging method for soft tissues, affording spatial resolution of 10 µm or less in three dimensions and has been widely applied in medicine, including ophthalmology, dermatology and dentistry [11–13]. In OCT, a light source is directed onto samples, and the time delay and intensity of the reflected light are used to construct a high-resolution image of the specimen. Here, we report the use of an OCT system to measure emulsion-based synthetic cells (Fig. 1) for the first time. The structural information of individual synthetic cells can be acquired efficiently without using additional water, or lipid soluble fluorescent dyes or fluorescently tagged biomolecules, such as proteins, peptides or oligonucleotides. It is also possible to use OCT systems for mapping tissue-like architectures made from synthetic cells, hence providing a cost-effective imaging tool for engineered compartmentalised soft structures.

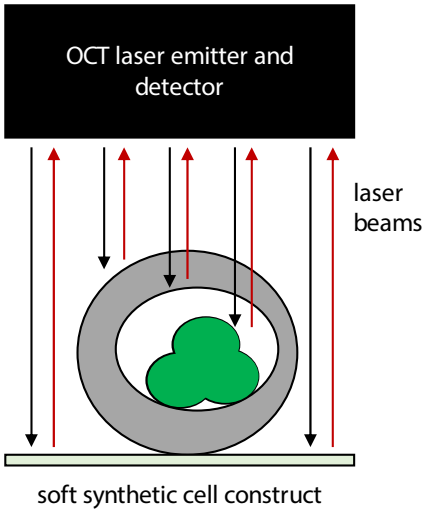

Fig. 1. Schematic of using optical coherence tomography (OCT) system to image a soft synthetic cell construct. The time delay and intensity of the reflected light are used to construct 3D images.

MATERIALS AND METHODS

OCT (VivoSight System, Michelson Diagnostics, UK) system was used to take axial images of synthetic cells. Synthetic cells were produced using 3D-printed microfluidic devices. Microfluidic device fabrication, chemical protocols and experiment setup can be found in our previous publications [6,14]. An example of multicompartment synthetic cell is shown in Fig. 2, which fluorescent image was taken using a EVOS M7000 microscope (Invitrogen). During the OCT imaging, synthetic cells were transferred onto a microscope glass slide, which was in turn placed on the imaging platform. The moving platform was adjusted to focus the microscope objective on the samples by tuning its z-axis dial. An area of 6 mm2 was scanned at 500 FPS with a 4 µm step size. Image processing was carried out using ImageJ Fiji, for the visualisation of orthogonal views and 3D image reconstruction of the synthetic cells.

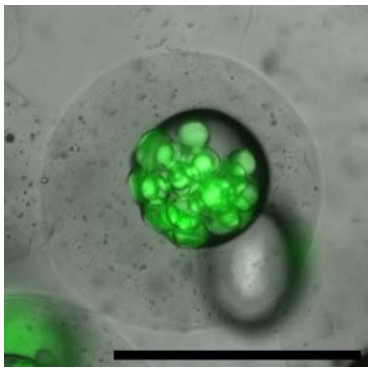

Fig. 2. Example of water (green)-in-oil-in- hydrogel synthetic cell. Scale bar = 500 µm.

RESULTS AND DISCUSSION

Free-standing, multicompartment synthetic cell capsules were made from water-in-oil-in-hydrogel-in oil complex emulsions (Fig. 2). The alginate shell hosts the fragile lipid-segregated droplet interface bilayer network inside [4], and provides the mechanical stability by virtue of which the synthetic cells can be manipulated and transported. The orthogonal views and z-projected image of an example synthetic cell is shown in Fig. 3. These orthogonal views can be used to extract data like the diameter of different compartments, and the contact angles of the droplet interface bilayers, informing on membrane forces. As shown in Fig. 4, 12 contact angle measurement can be implemented from a synthetic cell with a droplet network containing three compartments from a single OCT image, while with conventional 2D fluorescent imaging, this would be limited to 2 to 4 contact angle measurements, depending on the imaging orientation. These contact angle measurements can be used for non-destructive investigation of the artificial membrane properties inside synthetic cells. Regulation of membrane contact angle can be used to determine packing order [15], and therefore droplet network connectivity and function [16].

The volume of the three aqueous cores was calculated to be approximately 6, 7 and 8 nL, by utilizing the high contrast axial images, as shown in Fig. 5. This was achieved by measuring the area of each core at every axial scan and multiplying the sum of the area with the step resolution (4 µm). The thickest and the thinnest regions of the hydrogel shell layer can also be evaluated. The average hydrogel shell thickness (measured from all orthogonal views) of an individual artificial cell was estimated to be 340 µm ± 31 µm for the thickest, and 37 µm ± 3 µm for the thinnest in Fig. 5.

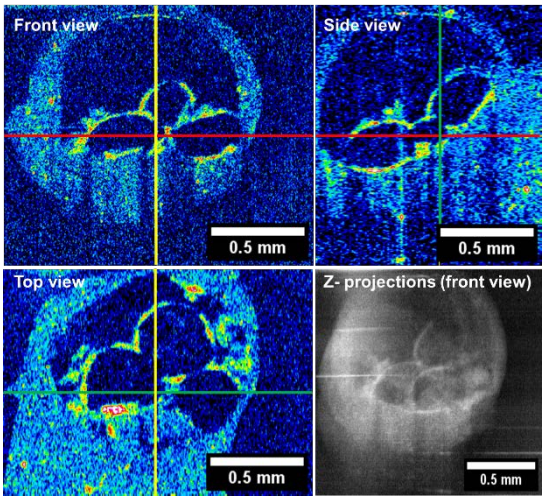

Fig. 3. Orthogonal views and z-projection of a synthetic cell imaged by OCT (with false 16-colour LUT).

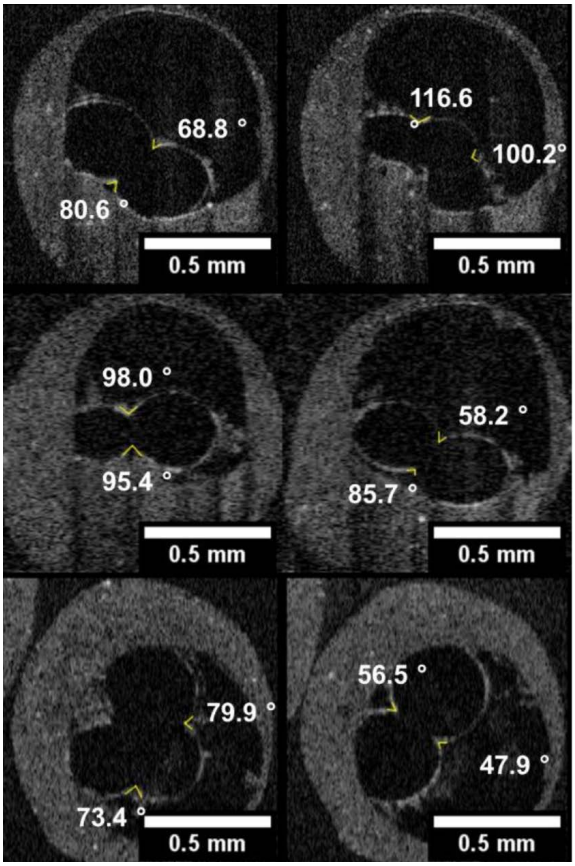

Fig. 4. OCT orthogonal views of a 3-core synthetic cell with 12 annotated contact angle values.

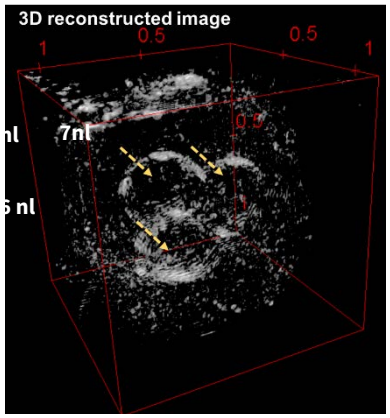

Fig. 5. 3D volumetric measurements of synthetic cell compartments of Fig. 4. The volumes of the three water compartments, highlighted by orange arrows, were calculated as 6, 7 and 8 nL.

Moreover, the capsule diameters can be measured from each orthogonal view (or from any desired angle of view) to identify the exact location of the compartment in three dimensions (3D). For example, the 3D central position (p3D) of the oil core (within which the aqueous droplet network in Fig. 5 is contained) was expressed as $P3_D$(centre) = 0.62x +0.66y+0.42z, by measuring the diameter of the synthetic cell outer shell in every axis (⌀x = 1.135 mm, ⌀y = 1.150 mm, ⌀z = 1.045 mm), considering the bottom left corner as the origin (x,y,z) = (0,0,0) (Fig. 6). Therefore, the approximate 3D structure of the synthetic cell can be mathematically reconstructed, providing numerical models for structural optimisation [17], and behavioural simulations [18], using COMSOL Multiphysics software.

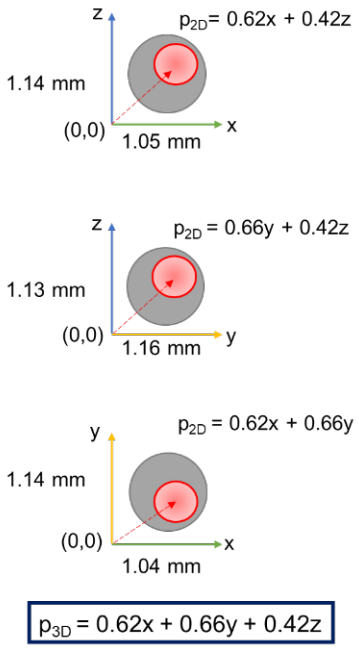

Fig. 6. Three-dimensional positions of sub-cellular oil compartment within the synthetic cell can be calculated as P3D, from the OCT imaging data of (i) front, (ii) side, and (iii) bottom.

In addition, the total imaging volume of the OCT system is sufficient to image large, tissue-like structures comprised of multiple artificial cells, that are not easily amenable to confocal microscopy imaging. As such, OCT is a suitable tool for the rapid scanning and mapping of 3D-printed superstructures of hybrid materials [19], for our research in the development and programming of functional synthetic tissues.

CONCLUSIONS

Optical coherence tomography can be utilised for label-free imaging of 3D architectures of bottom-up synthetic cells, and droplet-based soft materials. Volumetric information, spatial arrangements, and contact angles between sub-cellular compartments can be obtained from the OCT imaging data to assist the optimisation of synthetic cell fabrication.

Acknowledgments
This work was primarily supported and funded by the European Horizon 2020 project ACDC (Artificial Cells with Distributed Cores) under project award number 824060.

Conflicts of interest
The authors declare no conflict of interest.

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Cynhadledd Ymchwil Peirianneg Caerdydd
Cardiff Engineering Research Conference

E. Spezi and M. Bray (eds.) 2024. *Proceedings of the Cardiff University Engineering Research Conference 2023.* Cardiff: Cardiff University Press.
doi.org/10.18573/conf1

Cardiff University Engineering Research Conference 2023 was organised by the School of Engineering and held from 12 to 14 July 2023 at Cardiff University.

The work presented in these proceedings has been peer reviewed and approved by the conference organisers and associated scientific committee to ensure high academic standards have been met.

Cardiff University Press | Gwasg Prifysgol Caerdydd

First published 2024

Cardiff University Press
Cardiff University, PO Box 430
1st Floor, 30-36 Newport Road
Cardiff CF24 0DE

cardiffuniversitypress.org

Editorial design and layout by
Academic Visual Communication

ISBN: 978-1-9116-5349-3 (PDF)